COMPARING OTOLITHS, DORSAL SPINES, AND SCALES TO ESTIMATE AGE, GROWTH, AND MORTALITY BETWEEN MALE AND FEMALE WALLEYE FROM BROOKVILLE RESERVOIR, INDIANA

FINAL REPORT

STEVEN B. DONABAUER
ASSISTANT FISHERIES RESEARCH BIOLOGIST

FISHERIES SECTION
INDIANA DEPARTMENT OF NATURAL RESOURCES
INDIANA DIVISION OF FISH & WILDLIFE
I.G.C.-SOUTH, ROOM W 273
402 WEST WASHINGTON STREET
INDIANAPOLIS, IN 46204

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EXECUTIVE SUMMARY

- Walleye generate approximately $9.4 million annually in Indiana and a recent angler survey suggests respondents strongly support walleye stocking programs.
- Because Brookville Reservoir (Brookville) is Indiana's broodstock source for walleye, it presented an opportunity for the Division of Fish and Wildlife to collect age structure data on larger (and presumably older) fish that have been difficult to capture from northern waterbodies in Indiana.
- The goal of this study was to compare age structure statistics of walleye using whole otoliths, sectioned dorsal spines (i.e., standard method), and scales.
- The primary objectives were to determine: (1) precision; (2) bias; (3) age-frequency distributions, (4) growth rates, and (5) annual mortality rates.
- The secondary objectives were to: (6) differentiate true and false annuli by direct comparison among structures; and (7) refine the methods for transmitted light on un-sectioned dorsal spines (i.e., alternative method).
- Cumulative percent agreement among three age-analysts was highest for otoliths (77%), followed by dorsal spines (37%), and scales (20%); precision among age-analysts was slightly better for female walleye than for male walleye.
- Dorsal spines were under-aged relative to otoliths after age-6; scales were under-aged relative to otoliths after age-4; the degree of bias was greater for males.
- Age frequency distributions indicate that otoliths are an acceptable surrogate for known-age walleye in Brookville.
- Males grew significantly slower and had lower mortality rates than females.
- Interpretation of age-bias, age-histograms, growth rates, and cross-comparisons between dorsal spines and otoliths revealed that age-0+ check-marks in dorsal spines were occasionally misinterpreted as the age-1 annulus.
- An aging seminar should be directed by the research unit; the fisheries section should decide whether dorsal spines be adopted as the standard aging structure.
- No one statistical growth or mortality method is recommended at this time; all options should be reviewed by the section and standardized methods adopted.

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INTRODUCTION

Walleye *Stizostedion vitreum* are a coolwater species native to north-central North America (Pflieger 1997). In Indiana, walleye populations are maintained by stocking because habitat is generally inadequate for natural reproduction. This species is a highly-prized game fish that most anglers prefer to harvest given the excellent flavor of the fillets. Thus, walleye are economically important and generate approximately $9.4 million annually in Indiana (USFWS 2006). In a recent survey, walleye ranked as the seventh most popular sport fish among Indiana anglers only behind several species of native centrachids and ictalurids (Broussard and Haley 2005). Furthermore, survey respondents suggested the Division of Fish and Wildlife (DFW) stock walleye (25%) ahead of striped bass (22%), trout (18%), channel catfish (15%), muskellunge (8%), salmon (7%), and sauger (5%).

Most of the walleye fry and fingerlings stocked throughout Indiana by the DFW originate from annual broodstock collections at Brookville Reservoir (Brookville). The DFW's annual production costs are approximately $82,000 to raise walleye fry and fingerlings in State Fish Hatcheries (R. Lang, DFW, personal communication). Consequently, stocked walleye fisheries are regularly evaluated and management strategies adjusted to ensure the continued success of various walleye programs. Although evaluation criteria are often tailored to a specific waterbody, growth and mortality rate estimates are used as assessment tools by managers. Yet, interpretation of growth and mortality estimates depend largely on whether age data can be adequately determined from scale samples.

Calcified bony structures (otoliths, dorsal spines, and scales) of walleye have been compared by other researchers (Erickson 1983; Borkholder and Edwards 2001; Isermann et al. 2003) and among several Indiana waterbodies by the DFW including Crooked Lake (Steuben Co.; N = 31), Lake of the Woods (Marshall Co.; N = 71), Pike Lake (Kosciusko Co.; N = 9), and Kokomo Reservoir (Howard Co.; N = 33). However, since few large (Maximum = 19.6 in) or old fish (Maximum = age-5) were collected by the DFW, the results were inconsequential. Brookville offered a unique opportunity to collect gender-specific information on larger (> 20 in) and presumably older (age-6+) walleyes. The goal of this study was to compare age structure statistics of walleye using whole otoliths, sectioned dorsal spines (i.e., standard method), and scales collected during broodstock operations at Brookville in 2009. The primary objectives of this research were to use the age data to determine: (1) precision and (2) bias among calcified structures; and (3) age-frequency distributions, (4) growth rates, and (5) annual mortality rates between male and female walleye. The secondary objectives of this study were to: (6) differentiate true and false annuli based on direct post-concert cross comparisons among structures; and (7) refine the methods for transmitted light on un-sectioned dorsal spines (i.e., alternative method).

METHODS

Study area

Brookville is located in Franklin and Union counties in eastern Indiana (Figure 1). The reservoir was impounded in 1973-74 and the dam is controlled by the US Army Corps of Engineers. Brookville is the third largest reservoir in Indiana and has a surface area of 5,260 acres. The Division of Fish and Wildlife (DFW) has stocked walleye, striped bass, and muskellunge in Brookville on a regular basis to utilize the abundant gizzard shad population (Wisener 2003). The DFW conducts its annual walleye broodstock operations from late March to early April at Brookville. From 2000-08, the DFW's annual broodstock operations averaged (± SD) 13 (± 3) net nights to collect and spawn 428 (± 115) walleye, which yielded an average of 34 (± 5) million eggs per year. Because Brookville is Indiana's brood source for walleye, it is stocked annually with 10.5 million walleye fry. Most of the lakes and reservoirs that are stocked with walleye fry or fingerlings by the DFW originate from Brookville.

Field collection

Walleye were sampled at Brookville from 27 March to 8 April 2009. Two-hundred foot 2.25 in bar mesh gill nets (N = 128) were set parallel to the face of Brookville dam and perpendicular to it along the western shore in approximately 5 to 15 feet of water. Nets were set at approximately 1800 hours, checked and reset at 2400 hours, and then checked and pulled at 0800 hours. All walleye collected were sexed and all fish that were transported to Mounds Egg Taking Station (METS) were measured. Five male and female walleye from each half-inch length bin were sub-sampled for total length (± 0.1 in) and wet weight (± 1 oz). In addition, each sub-sampled fish had three aging structures removed: (1) approximately 12 scales between the lateral line and dorsal fin (DeVries and Frie 1996); (2) the first 3 anterior dorsal spines; and (3) both saggital otoliths. Aging structures were placed in serial numbered coin envelopes to be processed at a later date.

Laboratory process

All aging structures were allowed to air dry. Approximately six walleye scales per fish were heat pressed on acetate slides. A single (non-regenerated) scale impression of an acetate series was projected with a microfiche reader. A high resolution image (10.0 mega-pixel) of the projection was captured with a digital camera mounted on a tripod.

A low speed saw was used to make multiple 75 μm cross-sections (i.e., standard method) of the second anterior dorsal spine (third anterior dorsal spine used only if the second spine was not suitable for sectioning). All dorsal spine cross-sections and whole otoliths were placed against a black background, submerged in glycerin, and viewed with reflected light under a stereomicroscope. Digital images of the dorsal spines cross-sections and whole otoliths were captured with a trinocular-mounted camera and digital imaging software (SigmaScan Pro 5.0, Richmond, California).

Images of all calcified samples were uploaded into PowerPoint® (Microsoft Corporation, Redmond, Washington), which provided three age-analysts the ability to independently mark annuli without knowledge of fish length or age (i.e., pre-concert assignment). Age-analysts were aware that the fish were captured in the spring. After marking the annuli, independent analysts collectively resolved age discrepancies (i.e., post-concert consensus) and ages not agreed upon were excluded from further analysis. Analyst I had seven years of experience aging calcified structures, while Analyst II and III were inexperienced but received extensive training prior to the exercise.

Precision

Precision of pre-concert age assignments were estimated by: (1) cumulative percent agreement (*CPA*) tables; and (2) calculation of the coefficient of variation (*CV*) among independently assigned ages. The *CV* was calculated by:

$$CV_j = \frac{\sqrt{\sum_{i=1}^{R} \frac{(X_{ij} - X_j)^2}{n-1}}}{X_j} \cdot 100$$

where X_{ij} is the *i*th age estimate for the *j*th fish; X_j is the mean age estimate of the *j*th fish; and *n* is the number of times the fish was independently aged. The coefficient of variation was averaged across all fish to obtain mean *CV* values for each calcified structure.

The *CV* standardizes dispersion and is a measure of relative variation (i.e., percentage of the mean) contrary to the standard deviation (SD), which is a measure of absolute variation. For example, if two aging structures were compared and found to have the same SD regarding age assignments among analysts, one might falsely conclude that the age assignments between these structures are equally precise. However, if the mean ages among analysts are dissimilar among structures, the relative variability (i.e., *CV*) will show that precision is in fact unequal. Thus, the *CV* is a more useful statistic for comparing precision among calcified structures.

Bias

Age frequency tables were constructed to analyze age-analyst bias by comparing pre-concert age assignments to post-concert consensus. For example, if an analyst over-aged a walleye relative to post-concert consensus, then their pre-concert age assignment would be observed below the 1:1 diagonal illustrated in an age frequency table. Conversely, if an analyst under-aged a walleye relative to post-concert consensus, then their pre-concert age assignment would be represented above the 1:1 diagonal illustrated in an age frequency table. The number and degree of ages below (over-aged) or above (under-aged) the 1:1 consensus diagonal describes age-analyst bias.

Structural bias among calcified samples was analyzed with age-bias plots by pitting each consensus-aged calcified structure within individual walleyes against the other calcified structures. Theoretically, if all aging structures are unbiased, post-concert consensus ages among structures will have a 1:1 relationship. For example, an unbiased estimate would include post-concert consensus of an age-4 walleye regardless of the calcified structure used to estimate the age. Conversely, the degree of aging bias would be evident from data that deviate from the 1:1 consensus diagonal of an age-bias plot where an individual walleye would have a different age assignment among one or more calcified structures.

Age-frequency distributions

This study did not have access to known-age walleyes to validate the accuracy of age assignment among calcified structures. Thus, gender-specific age-length keys were used to expand the sub-sampled age data to fit the observed length frequency distributions. Age-length histograms were used to illustrate whether the age-specific distributions were realistic (i.e., normal distribution; Campana et al. 1995). The age frequency data were statistically compared ($\alpha = 0.10$) with the Kolmogorov-Smirnov asymptotic test statistic (*KSa*) that were Bonferroni-adjusted (0.10/9 = 0.011) to maintain the Type I error rate. All *KSa* comparisons were performed with Statistix 9.0 (Tallahassee, Florida).

Growth rates

Growth was estimated for pooled-gender data and separated by males and females. In addition, four techniques were used to estimate and compare growth rates: (1) mean length-at-capture derived from age-length keys; (2) back-calculated mean length-at-capture; (3) weighted mean back-calculated length-at-age; and (4) un-weighted mean back-calculated length-at-age data. The distances of consensus annular marks were measured with SigmaScan Pro 5.0 (Richmond, California) and growth was estimated in FishBC 2.0 (Ball State University, Muncie, Indiana) using 2.2 in as the intercept. Growth data were used to construct von Bertalanffy growth models in Fisheries Analysis and Simulation Tools (FAST 2.1; Auburn University, Auburn, Alabama):

$$l_t = L_\infty [1 - e^{-K(t+to)}]$$

where l_t is the fish length at time t, L_∞ is the theoretical maximum length for an average individual in the population, K is the Brody growth coefficient, and t_o is the correction factor to adjust for time when length is theoretically zero (Ricker 1975). The von Bertalanffy equations were linearized to solve for the time t (years) required for male and female walleye in Brookville to reach preferred size (20 in; Anderson and Neumann 1996), where L_∞ was held constant at 27 in and 32 in, respectively (R. Wisener, Indiana Department of Natural Resources, personal communication).

Annual mortality rates

The age frequency data were used to estimate total annual mortality A rates derived from Heincke's, Robson-Chapman's, and catch-curve regression techniques described by Miranda and Bettoli (2007). Ninety-five percent confidence intervals were generated and used to determine differences among calcified structures, statistical methods, and genders. Scenarios that produced over-lapping 95% confidence intervals were considered statistically similar.

Annuli identification

Two male walleye (fish 027 and 131) and two female walleye (fish 064 and 215) were randomly selected from the sub-sample to demonstrate post-concert cross-comparisons among calcified structures. The discrepancies among post-consensus marks were illustrated for each image to identify and differentiate true and false annuli.

Un-sectioned dorsal spines: refinement of an alternative method

In addition to the standard method for processing dorsal spines (i.e., cross-section, reflected light), an alternative method was also investigated. The skin was removed from the second dorsal spine of two males (fish 027 and 131) and two females (fish 064 and 215). The basal end of the spine was polished with a fine-grit (2,000 grit) sand paper. Each un-sectioned spine was imbedded into modeling clay and two fiber-optic terminals transmitted light through the basal end of the spine (Figure 2). Digital images of un-sectioned spines were captured with a trinocular-mounted camera and digital imaging software (SigmaScan Pro 5.0, Richmond, California). The images produced by the alternative method were directly compared to the images of the standard method to determine the advantages and limitations of each method.

RESULTS

Walleye (701 males and 815 females) were collected during broodstock operations at Brookville in 2009; total length data were collected from 368 males and 591 females; calcified structures were sub-sampled from 117 males, 135 females, and 2 walleye of unknown gender. Scales were removed from all sub-sampled walleye and dorsal spines and otoliths were removed from all but 2 individuals (N = 252, respectively). Median (range) total length (TL) for sub-sampled walleye was 19.1 in (13.0 to 24.3 in) for males and 21.9 in (17.5 to 27.9 in) for females. Median wet weight (WW) of sub-sampled walleye was 2.5 lbs (0.7 to 5.3 lbs) for males and 3.8 (1.6 to 8.2 lbs) for females. The length (in)–weight (oz) relationships for walleye were described by linear regressions for males $Log_{10}\ WW_M = 3.351(Log_{10}\ TL_M) - 2.708$ (N = 117; r^2 = 0.982), females $Log_{10}\ WW_F = 3.106(Log_{10}\ TL_F) - 2.388$ (N = 135; r^2 = 0.926), and for pooled-gender walleye $Log_{10}\ WW_P = 3.224(Log_{10}\ TL_P) - 2.546$ (N = 254; r^2 = 0.974). Post-concert consensus was reached among all (100%) walleye otoliths, 249 (99%) dorsal spines, and 246 (97%) scales.

Precision

When gender data were pooled, pre-concert *CPA* (± 0 years) among all age-analysts was much higher (77%) for otoliths than either dorsal spines (37%) or scales (20%; Table 1). Cumulative percent agreement (± 1 year) among pooled-gender walleye increased for otoliths (96%), dorsal spines (83%) and scales (66%; Table 1). Higher *CPA* values and lower *CV* values indicated that age-analysts were slightly more precise when assigning ages to females regardless of the calcified structure used to estimate age (Table 1).

Bias

Pre-concert age comparisons between individual age-analysts and post-concert consensus for scales revealed that age-analyst I was unbiased (Table 2), whereas age-analyst II (Table 3) and age-analyst III (Table 4) over-aged walleye to age-4 and under-aged older (age-5+) walleye. Age-analyst I under-aged dorsal spines for walleye ages-4+, however most (78%) of the deviation from consensus was under-aged by 1 year (Table 5). Age-analyst II also under-aged dorsal spines for walleye ages-4+ and again most (77%) of the deviation from consensus was explained by 1 year (Table 6). Age-analyst III over-aged dorsal spines for walleye to age-3, was unbiased for ages 4-5, and under-aged older (age-6+) age-classes (Table 7). Age-analysts I (Table 8), II (Table 9), and III (Table 10) slightly over-aged otoliths for walleye to age-3, were unbiased for age-4 walleye, and slightly under-aged older (age 5+) age classes.

Post-concert age-bias plots indicated that dorsal spines were generally unbiased for young (< age-6) walleye but under-aged older (age-7+) walleye when compared to otoliths. This trend was more exaggerated in males (Figure 3). Scales were generally unbiased when compared to otoliths for walleye up to age-4, but were under-aged compared to otoliths for older (age-5+) aged walleye (Figure 4). Again, this trend was more pronounced in males. Furthermore, the under-aging bias was more extreme for scales than for dorsal spines when both structures were compared to otoliths. For example, when male walleyes were aged to be 7 years with otoliths, the mean ages assigned to scales and dorsal spines were 4.6 and 6.4 years, respectively. When scales were directly compared to dorsal spines (Figure 5), they tended to under-age walleye ages-4+ and again the bias was more severe for males.

Age-frequency distributions

The age-length histograms revealed that otoliths provided the most plausible age-specific distributions for male and female walleye. Most of the age-specific distributions derived from otoliths were normally distributed for both genders and the tails of each distribution were more narrowly defined. There were a few exceptions to this generality for male walleye. For example, age-4 males aged with otoliths had a bi-modal distribution and age-5 and age-6 were positively skewed. The age-specific distributions derived from dorsal spines more closely resembled otolith than scale distributions. However, both male (Figure 6) and female (Figure 7) age-5 walleye showed bi-modal distributions when derived from dorsal spines. Age distributions derived from scales were the most unrealistic. For example, the age-length distribution for age-3 males and females included fish from 13.5 to 22.5 in and 17.5 to 26.5 in, respectively. Moreover, the age-length distribution for age-5 females was negatively skewed and encompassed fish from 17.5 to 25.0. Overall, the age-specific distributions were more difficult to interpret among males because the distributions crowded together. When otoliths were statistically compared to dorsal spines, the cumulative age-frequency distributions (Figure 8) were not significantly different from each other for males (KSa = 0.09, N = 227, P = 0.74), females (KSa = 0.04, N = 270, P = 1.0), or pooled-gender data (KSa = 0.06, N = 501, P = 0.72). Age distributions derived from scales were significantly different (Table 11) than those derived from dorsal spines and otoliths for males and pooled-gender data but were not significantly different for females.

<u>Growth rates</u>

Growth rates derived from dorsal spines generally had lower length-at-age estimates than either otoliths or scales regardless of the method (age-length key mean length at-capture [Table 12], back-calculated mean length-at-capture [Table 13], weighted mean back-calculated length [Table 14], or un-weighted mean back calculated length [Table 15]) or gender (pooled-gender, male, or female) used to derive the estimate. Weighted and un-weighted back-calculated methods revealed that growth estimates derived by otoliths yielded higher growth estimates for younger (< age-3) walleye than estimates derived by dorsal spines and scales. Yet, scales typically had higher growth rate estimates for older (age-4+) walleye among statistical methods. The growth of males slowed substantially after age-5 (approximately 21 in TL), whereas female growth slowed considerably after age-6 (approximately 26 in TL). The time required for walleye to reach preferred size (20 in) was generally higher for estimates derived from mean length-at-capture data (male range: 6.5 to 12.1 years; female range: 5.9 to 8.8 years) than from estimates derived from back-calculated methods (male range: 5.9 to 9.2 years;

female range 4.5 to 6.0 years) and were consistently lower when estimated with scales (male range: 5.9 to 8.1 years; female range: 4.5 to 6.5 years) than for either dorsal spines (male range: 6.9 to 12.1 years; female range: 4.9 to 8.8 years) or otoliths (male range: 8.0 to 10.6 years; female range: 5.9 to 7.2 years; Table 16).

Annual mortality rates

All dorsal spine annual mortality scenarios were similar (over-lapping 95% confidence intervals) to otoliths when the same method (i.e., Heicke's, Robson-Chapman's, catch-curve) was used to derive the estimates (Table 17). Among calcified structures, scales produced higher annual mortality estimates than either otoliths or dorsal spines. Among statistical methods, catch-curves produced higher estimates than Heicke's or Robson Chapman's methods. Although Heicke's method produced the lowest estimates, the 95% confidence intervals generally over-lapped with estimates derived by the Robson-Chapman's method. Overall, males had lower annual mortality rates than females regardless of the calcified structure or statistical method used to derive the estimate.

Annuli identification

Direct post-concert cross comparisons of calcified structures within the same walleyes were conducted for male 027 (Figures 9a-d), female 064 (Figures 10a-d), male 131 (Figures 11a-d), and female 215 (Figures 12a-d). The most important finding was the identification of an age-0+ check-mark observed among dorsal spines, that when properly identified, reconciled most of the age discrepancies between the dorsal spines and otoliths. When discrepancies beyond the age-0+ check-mark persisted, other inconsistencies including double-banding (Figure 10b), and false annuli (Figure 11b) were identified due to comparatively weaker bands than observed among true annuli. Annuli were difficult to interpret among scales of older (age-5+) walleye because the cross-over patterns were increasingly strenuous to identify along the lateral margins.

Un-sectioned dorsal spines: refinement of an alternative method

The alternative method of transmitted light through un-sectioned dorsal spines was successfully achieved in walleyes 027 (Figure 9c), 064 (Figure 10c), 131 (Figure 11c), and 215 (Figure 12c). The most obvious difference between the alternative method and standard method is that the color patterns that define the annuli and growth periods are reversed (i.e., annuli are light rather than dark, and growth is dark rather than light). All of the features evident in the standard method (i.e., age-0+ check-marks, double-banding, and false annuli) were also observed using the alternative method.

DISCUSSION

Otoliths: a sufficient surrogate for known-age walleye

The only way to assess aging bias with strict confidence is to incorporate known-age fish into a study design. A vigorous mark-recapture study would provide the requisite data. However, the most useful information (i.e., old fish) from a mark-recapture design is difficult to obtain across the temporal scale because the longer a marked individual is *at-large* the more challenging it is to recapture (e.g., emigration, mortality, size-selective capture vulnerability, etc.). Consequently, this study used an alternative technique to indirectly validate the accuracy of calcified structures by analyzing age-length histograms based on consensus (post-concert) age assignment. Theoretically, each year-class is normally distributed where the mean length of successive year-classes increases while the frequency of each year-class decreases. The data obtained in this study revealed that age data derived from otoliths most closely resembled theoretical age-length distributions for male and female walleye and thereby validated the accuracy of this aging structure. Therefore, otoliths were considered an acceptable surrogate for known-age walleye from Brookville.

Identification of age-0+ check-marks: confidence in age-1 assignment of dorsal spines

Interpretation of age-bias plots, age-length histograms, back-calculated lengths, and direct cross-comparisons between dorsal spines and otoliths revealed that the age-0+ check-marks in dorsal spines were occasionally misinterpreted by age-analysts.

Although the most obvious trend among age-bias plots for dorsal spines revealed that older-aged (age-6+) walleye were under-aged, close inspection also indicates a slight tendency to over-age young (up to age-4) walleye. This subtle bias provides evidence that age-analysts occasionally misinterpreted the age-0+ check-mark(s) in dorsal spines as the first annulus. This error also manifested itself among the age-length histograms where bi-modal distributions for dorsal spines were apparent for age-5 male and female walleye. Additionally, the age-specific distributions among older (age-6+) aged walleye were negatively skewed for dorsal spines when compared to otolith distributions, indicative of a systematic over-aging bias among comparatively smaller walleye. Analysis of back-calculated length data further indicates that the age-0+ check-mark(s) were incorrectly marked as the first annulus. For example, un-weighted mean back-calculated lengths for age-1 males and females were higher when estimated with otoliths (11.0 and 12.0 in, respectively) than when estimated with dorsal spines (8.3 and 8.7 in, respectively). Had the age-0+ check-mark(s) and first annulus been correctly identified during post-concert consensus among all dorsal spines, it is reasonable to suspect that the un-weighted mean back-calculated lengths for age-1 walleye would have increased and better approximated the estimates derived from otoliths. Perhaps the most convincing evidence that the age-0+ check-mark(s) were not true annuli is provided by the direct cross-comparisons of digital images between dorsal spines and otoliths. Multiple age-0+ check-marks were frequently observed among individual dorsal spine samples. The cause of age-0+ check-marks is open to debate. Perhaps age-0+ check-marks are related to the stress of the stocking period or when the walleye transition from endogenous to exogenous feeding. Typically, the age-0+ check-mark(s) are not as well defined as the age-1 annulus among dorsal spines. However, walleye 215 presents an exception to this generality where the age-0+ check-mark was so well defined that it was essentially indistinguishable from true annuli (Figure 12b). This presents a problem for aging individual walleye accurately with dorsal spines when direct cross-comparisons to the otoliths are not an option. However, the cumulative frequency distributions between dorsal spines and otoliths revealed that such discrepancies are a statistically insignificant (*KSa* tests) problem given sufficient sample sizes.

Knowledge of length (or sorted samples) prior to age assignment

The growth rates for walleye from Brookville support the view that age-analysts should not have knowledge of fish gender, length, or sort samples (e.g., by length) prior to age assignment. Gender information is usually not collected during routine surveys so prior knowledge of this information is typically not a concern. As a consequence, however, there is no way to assess gender differences. Male and female walleye from Brookville were shown to have different growth rates in this study. Thus, knowledge of length prior to age-assignment is ill-advised because it increases the likelihood of an age-analyst committing Type II error (i.e., concluding that there is not a difference between factors; when in reality – there is a difference). Consider the following *thought-experiment*. Assume an investigator is charged to determine whether faster-growing walleyes will result if the annual stocking rate is decreased. The null hypothesis would state that there is no difference in the growth of walleye between stocking rates. Next, the investigator collects a sample of walleye before and after the stocking rate change and proceeds to analyze sorted calcified structures with knowledge of individual walleye lengths prior to age-assignment. Under this scenario, the investigators preconceived notions will result in incremental growth patterns that are more similar than they are dissimilar because the investigator was influenced (either consciously or sub-consciously) by prior knowledge and thus committed Type II error. Such biases lead the investigator to fail to reject the null hypothesis and will result in an erroneous conclusion (i.e., stocking rate had no influence on growth rates; when in reality – a decrease in the stocking rate did increase growth rates). Even worse, recommendations may be proposed and initiated that are detrimental to the fishery (e.g., stock walleye at the higher rate; when in reality – the stocking rate should remain at a lower rate if higher growth rates are desired). Many of the problems associated with the principles of erroneous conclusions (Brown and Guy 2007) can be avoided by randomizing samples, avoiding temptations to read individual length prior to age-assignment, and using consistent, objective, vetted criteria to identify annuli.

Growth and mortality methods: future standards

The data from Brookville indicated that male and female walleye were not fully recruited to the gill nets until they reached age-3 and age-4, respectively. It is not unusual for young fish (< age-3) to be under-represented during routine fish surveys and it has proven challenging to collect larger (and presumably older) walleye in the glacial lakes of northern Indiana (J. Pearson, Indiana DNR, personal communication). Such limitations should be considered when deciding what statistical method is appropriate to evaluate a management objective. The gill nets used at Brookville in this study are not the standard sampling gear used to evaluate walleye stocking programs in Indiana and likely played a significant role in the low catch of younger walleye. Nevertheless, this study demonstrated that growth and mortality estimates can vary substantially depending on the calcified structure or statistical method used to derive the estimate. Without alternative field methods (e.g., mark-recapture) to compare estimates, it is impossible to address the accuracy of each method. Thus, it is difficult to recommend the adoption of one statistical method over another at this time.

All of the annual mortality statistics generated in this study were reliant on the assumptions of constant recruitment, constant mortality, and equal capture vulnerability. Constant recruitment of age-0 walleye is a reasonable assumption assuming that 10.5 million fry (2,000/ac) are stocked annually with no natural reproduction occurring in Brookville. Constant mortality is probably not a valid assumption due to natural environmental fluctuations; however, data collected over successive years would provide sufficient estimates across the temporal scale. Equal capture vulnerability is of concern as previously discussed. Yet, because recruitment of age-0 walleye can be considered constant in Brookville, mortality estimates could be used to determine the time required to reduce an age-specific segment of the population to a specified number of individuals per unit area (e.g., *x* number of age-*y* walleye/acre).

Accurate age estimates coupled with population estimates would provide an approach to validate mortality estimates. Consider another *thought-experiment*. Imagine an investigator estimates annual mortality rates for a walleye population using Heicke's, Robson-Chapman's, and catch-curve methods to be 40%, 50%, and 60%, respectively. The investigator then applies these estimates to known annual stocking rates (2,000/ac) and determines the time required for each estimated mortality rate to reduce a year-class to 100 fish per acre is 5.9, 4.4 and 3.3 years, respectively. To validate which statistical mortality estimate provides the most accurate results, the investigator also conducted an age-specific population estimate and found 100 age-4 walleye per acre. Thus, it would be concluded that that the Robson-Chapman method produced the most accurate mortality estimates. Had 100 age-3 or 100 age-6 walleye been collected per acre, either catch-curve or Heicke's method, respectively, would have been deemed more accurate than alternative methods. If the fisheries section is interested in using mortality as an evaluation tool, the accuracy among available statistical methods should be addressed so that a standardized method can be adopted and justified.

<u>The alternative method for processing dorsal spines</u>

This study has shown that both the standard and alternative methods for imaging dorsal spines are effective. Other researchers (Logsdon 2007) and state agencies (M. Mylchreest, Michigan DNR, personal communication) use transmitted light to age walleye dorsal spines. So, the question is – which method should be adopted as standard practice in Indiana? I found advantages (and disadvantages) for either technique. Standard method advantages include: (1) the skin of the dorsal spines do not have to be removed as a time-saving measure, and (2) it is a fairly straightforward process to capture an image with reflected light because the samples are not as sensitive to the precise adjustments of the fiberoptic terminals. The alternative method advantages include: (1) dorsal spines can be polished with fine-grit sand paper rather than being sectioned by a low-speed Isomet saw that may be cost prohibitive for each district office, and (2) true annuli appear to be slightly more distinct relative to false annuli (Figures 12b and 12c).

<u>Management implications</u>

Walleye are arguably the most important sport fish stocked in Indiana waters. Proper evaluation procedures for waterbodies containing walleye are critical if such stocking programs are to be successful. This paper has identified dorsal spines as a superior non-lethal aging technique that should be used to aid in the evaluation of walleye stocking programs. Furthermore, it is also encouraged that biologists randomize their samples and assign ages without knowledge of fish length. Collectively, these procedures will increase consistency, reduce systematic biases, and allow managers to draw conclusions derived from reliable empirical data that aim to maximize the potential of walleye fisheries and angler satisfaction in Indiana.

RECOMMENDATIONS

- The north research unit should lead a walleye aging seminar for DFW fish management biologists who will collectively decide whether dorsal spines be adopted (or rejected) as the standard aging structure for walleye evaluations in Indiana.
- The fisheries section should review statistical methods regarding growth and mortality and collectively decide whether the adoption (or rejection) of specific methods is warranted in an effort to set consistent evaluation criteria for workplan or strategic objectives.
- Regardless of the calcified structure used to estimate age, managers are encouraged to avoid sorting samples (e.g., by length) or have knowledge of the length of individual fish prior to age assignment to circumvent Type II error.

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APPROVAL

Submitted by: Steven B. Donabauer
Assistant Research Biologist
Submitted: December 13, 2010

Approved by: Stuart T. Shipman
North Region Supervisor
Approved: January 4, 2011

Table 1.—Cumulative percent agreement (± years) among three age-analysts and mean percent coefficient of variation (*CV*) for walleye collected at Brookville Reservoir in 2009. Data are delineated by aging structure and gender.

	N	Percent Agreement (%)					*CV* (%)
		Exact	± 1 yr	± 2 yr	± 3 yr	> 4 yr	
Pooled-gender							
Scale	254	20	66	92	100	100	16.4
Dorsal Spine	252	37	83	98	99	100	11.2
Otolith	252	77	96	99	99	100	3.6
Males							
Scale	117	16	68	93	100	100	18.1
Dorsal Spine	115	29	77	98	100	100	13.6
Otolith	115	70	93	100	100	100	4.5
Females							
Scale	135	25	65	94	100	100	14.8
Dorsal Spine	135	46	89	98	99	100	8.8
Otolith	135	84	100	100	100	100	2.1

Table 2.—Scale age comparisons for Brookville Reservoir walleye between age-analyst I and consensus ages among three age-analysts. Bold numbers represent 1:1 agreement. Data are numbers of fish.

		Post-concert Scale Age Consensus										
Pre-concert Age Assignment (Age-analyst I)	Age (yrs)	0	1	2	3	4	5	6	7	8	9	10
	0											
	1		**1**									
	2			**16**	5	1	1					
	3			1	**44**	17	2					
	4				15	**58**	13	4				
	5				3	14	**16**	3	1			
	6					3	15	**6**				
	7						1	4				
	8						1	1				
	9											
	10											

Table 3.—Scale age comparisons for Brookville Reservoir walleye between age-analyst II and consensus ages among three age-analysts. Bold numbers represent 1:1 agreement. Data are numbers of fish.

		Post-concert Scale Age Consensus										
	Age (yrs)	0	1	2	3	4	5	6	7	8	9	10
Pre-concert Age Assignment (Age-analyst II)	0											
	1											
	2		1	**6**								
	3			9	**49**	6		1				
	4			2	14	**74**	14	3				
	5				3	10	**29**	5	1			
	6				1	1	5	**8**				
	7					2	1	1				
	8											
	9											
	10											

Table 4.—Scale age comparisons for Brookville Reservoir walleye between age-analyst III and consensus ages among three age-analysts. Bold numbers represent 1:1 agreement. Data are numbers of fish.

		Post-concert Scale Age Consensus										
Pre-concert Age Assignment (Age-analyst III)	Age (yrs)	0	1	2	3	4	5	6	7	8	9	10
	0											
	1											
	2			**6**	5							
	3		1	5	**28**	13	9	1	1			
	4			6	28	**56**	10	4				
	5				5	17	**22**	3				
	6				1	6	6	**8**				
	7					1	2	2				
	8											
	9											
	10											

Table 5.—Dorsal spine age comparisons for Brookville Reservoir walleye between age-analyst I and consensus ages among three age-analysts. Bold numbers represent 1:1 agreement. Data are numbers of fish.

		Post-concert Dorsal Spine Age Consensus										
Pre-concert Age Assignment (Age-analyst I)	Age (yrs)	0	1	2	3	4	5	6	7	8	9	10
	0											
	1		**2**									
	2			**12**	2							
	3				**45**	15	4					
	4				2	**64**	13	2	1			
	5					3	**32**	6				
	6				1			**17**	9	2		
	7							1	**14**	2		
	8											
	9											
	10											

Table 6.—Dorsal spine age comparisons for Brookville Reservoir walleye between age-analyst II and consensus ages among three age-analysts. Bold numbers represent 1:1 agreement. Data are numbers of fish.

		Post-concert Dorsal Spine Age Consensus										
	Age (yrs)	0	1	2	3	4	5	6	7	8	9	10
Pre-concert Age Assignment (Age-analyst II)	0											
	1											
	2		2	**10**	8	1						
	3			1	**37**	31	7	2				
	4			1	3	**49**	13	2				
	5				1	1	**29**	10	3			
	6				1			**10**	7	2		
	7							2	**14**	1		
	8									**1**		
	9											
	10											

Table 7.—Dorsal spine age comparisons for Brookville Reservoir walleye between age-analyst III and consensus ages among three age-analysts. Bold numbers represent 1:1 agreement. Data are numbers of fish.

		Post-concert Dorsal Spine Age Consensus										
	Age (yrs)	0	1	2	3	4	5	6	7	8	9	10
Pre-concert Age Assignment (Age-analyst III)	0											
	1											
	2		2	**7**	1							
	3			5	**31**	9	1		1			
	4				16	**67**	9	4				
	5				1	6	**30**	10	7			
	6				1		8	**12**	6			
	7						1		**10**	3		
	8									**1**		
	9											
	10											

Table 8.—Otolith age comparisons for Brookville Reservoir walleye between age-analyst I and consensus ages among three age-analysts. Bold numbers represent 1:1 agreement. Data are numbers of fish.

		Post-concert Otolith Age Consensus										
	Age (yrs)	0	1	2	3	4	5	6	7	8	9	10
Pre-concert Age Assignment (Age-analyst I)	0											
	1		**2**									
	2			**19**								
	3			2	**54**	1						
	4				5	**67**	2	1				
	5					1	**45**	3				
	6						1	**24**	3	1		
	7							1	**15**			
	8									**3**		
	9									2		
	10											

Table 9.—Otolith age comparisons for Brookville Reservoir walleye between age-analyst II and consensus ages among three age-analysts. Bold numbers represent 1:1 agreement. Data are numbers of fish.

		Post-concert Otolith Age Consensus										
Pre-concert Age Assignment (Age-analyst II)	Age (yrs)	0	1	2	3	4	5	6	7	8	9	10
	0											
	1		**2**									
	2			**21**								
	3				**57**	1						
	4				2	**67**	1	1				
	5					1	**47**	4	2			
	6							**24**	3			
	7								**13**	4		
	8									**2**		
	9											
	10											

Table 10.—Otolith age comparisons for Brookville Reservoir walleye between age-analyst III and consensus ages among three age-analysts. Bold numbers represent 1:1 agreement. Data are numbers of fish.

		Post-concert Otolith Age Consensus										
Pre-concert Age Assignment (Age-analyst III)	Age (yrs)	0	1	2	3	4	5	6	7	8	9	10
	0											
	1		**2**									
	2			**15**	1			1				
	3			5	**54**	5						
	4				4	**60**	8					
	5			1		4	**39**	3				
	6						1	**25**	2			
	7								**15**	3		
	8								1	**3**		
	9											
	10											

Table 11.—Kolmogorov-Smirnov pairwise age distribution comparison of walleye collected at Brookville Reservoir in 2009. Calcified structures by gender are denoted by OP = otolith pooled, DP = dorsal spine pooled, SP = scale pooled, OM = otolith male, DM = dorsal spine male, SM = scale male, OF = otolith female, DF = dorsal spine female, and SF = scale female. N is the number of sub-sampled walleye compared. *KSa* is the Kolmogorov-Smirnov asymptotic test statistic that was Bonferroni adjusted (0.10/9 = 0.011) to maintain the type I error rate. Statistical significance ($\alpha = 0.10$) was denoted by * while a non-significant result was indicated by NS. Graphical representations of comparisons in this table are illustrated in Figure 8.

Comparison	N	*KSa* Statistic	*P*-value	Statistical significance
OP vs DP	501	0.06	0.719	NS
OP vs SP	498	0.14	0.030	*
DP vs SP	495	0.14	0.023	*
OM vs DM	227	0.09	0.736	NS
OM vs SM	230	0.18	0.054	*
DM vs SM	227	0.18	0.061	*
OF vs DF	270	0.04	1.000	NS
OF vs SF	264	0.12	0.308	NS
DF vs SF	264	0.12	0.308	NS

Table 12.—Mean (± SE) length-at-capture for walleye (pooled-gender and separated by males and females) derived from age-length keys using otoliths, dorsal spines, and scales collected at Brookville Reservoir, 2009.

	Otoliths			Dorsal Spines			Scales		
Age	Mean (in)	SE (in)	N	Mean (in)	SE (in)	N	Mean (in)	SE (in)	N
Pooled-gender									
1	-	-	-	-	-	-	-	-	-
2	14.7	0.2	24	14.1	0.1	14	15.7	0.7	18
3	19.1	0.1	230	19.2	0.1	196	19.4	0.1	262
4	20.7	0.1	322	20.6	0.1	360	20.9	0.1	422
5	21.7	0.1	213	21.4	0.1	217	22.0	0.2	203
6	22.4	0.2	106	22.1	0.2	95	23.3	0.3	52
7	23.5	0.7	50	23.4	0.3	70	26.8	0.0	2
8	23.3	0.9	14	24.4	1.0	7	-	-	-
Males									
1	-	-	-	-	-	-	-	-	-
2	14.7	0.2	24	14.1	0.1	14	14.3	0.2	15
3	18.6	0.1	140	18.4	0.1	100	18.5	0.1	148
4	19.2	0.1	80	19.1	0.1	102	19.5	0.1	106
5	20.3	0.1	64	19.8	0.2	79	20.6	0.1	77
6	21.5	0.2	41	20.9	0.2	40	21.3	0.3	22
7	21.5	0.2	18	21.2	0.2	31	-	-	-
8	23.3	-	1	21.8	-	2	-	-	-
Females									
1	-	-	-	-	-	-	-	-	-
2	-	-	-	-	-	-	21.8	0.0	5
3	19.9	0.1	120	19.9	0.1	101	20.3	0.1	126
4	21.1	0.1	303	21.4	0.1	293	21.5	0.1	345
5	23.2	0.1	120	22.2	0.2	138	23.5	0.2	93
6	25.5	0.2	21	24.3	0.3	27	26.0	0.2	20
7	25.9	0.2	22	25.9	0.2	28	26.8	0.0	2
8	27.3	0.2	5	26.7	0.5	4	-	-	-

Table 13.—Mean (± SE) back-calculated length-at-capture for walleye (pooled-gender and separated by males and females) using otoliths, dorsal spines, and scales collected at Brookville Reservoir, 2009.

	Otoliths			Dorsal Spines			Scales		
Age	Mean (in)	SE (in)	N	Mean (in)	SE (in)	N	Mean (in)	SE (in)	N
Pooled-gender									
1	9.5	0.1	2	9.5	0.1	2	9.6	0.0	1
2	14.4	0.2	21	14.2	0.2	12	14.4	0.6	17
3	18.5	0.2	57	18.3	0.2	49	18.6	0.3	65
4	20.7	0.2	69	20.6	0.2	82	21.0	0.2	93
5	22.2	0.3	49	21.6	0.4	49	22.5	0.4	51
6	23.2	0.4	29	23.0	0.4	26	24.4	0.6	17
7	24.7	0.6	18	24.2	0.6	24	26.8	0.0	1
8	25.0	1.2	6	25.6	1.4	4	-	-	-
Males									
1	-	-	-	-	-	-	-	-	-
2	14.4	0.2	21	14.2	0.2	12	14.2	0.2	15
3	17.9	0.2	33	17.6	0.3	30	17.9	0.3	43
4	19.2	0.3	15	18.8	0.3	23	20.1	0.4	26
5	20.5	0.3	18	20.0	0.4	20	20.9	0.3	24
6	21.9	0.3	19	21.7	0.3	16	22.0	0.6	7
7	21.8	0.4	7	21.4	0.7	10	-	-	-
8	21.6	1.2	2	21.6	0.0	1	-	-	-
Females									
1	-	-	-	-	-	-	-	-	-
2	-	-	-	-	-	-	21.7	0.0	1
3	19.3	0.2	24	19.3	0.3	19	20.1	0.4	22
4	21.1	0.2	54	21.2	0.2	59	21.4	0.2	67
5	23.1	0.3	31	22.8	0.4	29	24.0	0.5	27
6	25.7	0.3	10	25.0	0.5	10	26.1	0.4	10
7	26.5	0.2	11	26.2	0.2	14	26.8	0.0	1
8	26.7	0.6	4	26.9	0.7	3	-	-	-

Table 14.—Weighted mean (± SE) back-calculated length-at-age for walleye (pooled-gender and separated by males and females) using otoliths, dorsal spines, and scales collected at Brookville Reservoir, 2009.

	Otoliths			Dorsal Spines			Scales		
Age	Mean (in)	SE (in)	N	Mean (in)	SE (in)	N	Mean (in)	SE (in)	N
Pooled-gender									
1	11.5	0.1	251	8.7	0.1	248	10.3	0.1	245
2	16.0	0.1	249	13.8	0.1	246	15.2	0.1	244
3	18.9	0.1	228	17.2	0.1	234	18.6	0.1	227
4	20.8	0.1	171	19.8	0.2	185	20.9	0.2	162
5	22.3	0.2	102	21.3	0.2	103	22.7	0.3	69
6	23.4	0.3	53	22.8	0.3	54	24.5	0.6	18
7	24.6	0.5	24	24.3	0.5	28	26.8	0.0	1
8	25.0	1.2	6	25.6	1.4	4	-	-	-
Males									
1	11.1	0.1	115	8.4	0.2	112	10.0	0.2	115
2	15.4	0.1	115	13.1	0.2	112	14.6	0.2	115
3	17.8	0.1	94	16.1	0.2	100	17.6	0.2	100
4	19.4	0.1	61	18.0	0.2	70	19.6	0.2	57
5	20.6	0.2	46	19.6	0.3	47	20.9	0.3	31
6	21.6	0.2	28	21.0	0.3	27	22.0	0.6	7
7	21.6	0.4	9	21.4	0.6	11	-	-	-
8	21.6	1.2	2	21.6	0.0	1	-	-	-
Females									
1	11.8	0.1	134	8.9	0.1	134	10.6	0.2	128
2	16.6	0.1	134	14.3	0.2	134	15.7	0.2	128
3	19.8	0.1	134	18.1	0.2	134	19.3	0.2	127
4	21.7	0.1	110	20.8	0.2	115	21.7	0.2	105
5	23.7	0.2	56	22.7	0.3	56	24.1	0.4	38
6	25.6	0.2	25	24.6	0.3	27	26.1	0.3	11
7	26.3	0.2	15	26.1	0.2	17	26.8	0.0	1
8	26.7	0.6	4	26.9	0.7	3	-	-	-

Table 15.—Un-weighted mean (± SE) back-calculated length-at-age for walleye (pooled-gender and separated by males and females) using otoliths, dorsal spines, and scales collected at Brookville Reservoir, 2009.

	Otoliths			Dorsal Spines			Scales		
Age	Mean (in)	SE (in)	N	Mean (in)	SE (in)	N	Mean (in)	SE (in)	N
Pooled-gender									
1	11.5	0.3	7	8.4	0.3	7	10.3	0.3	5
2	16.0	0.3	7	13.4	0.4	7	15.0	0.3	5
3	18.9	0.6	6	16.7	2.1	6	18.5	0.3	4
4	20.9	0.5	5	19.2	0.5	5	20.9	0.3	3
5	22.4	0.6	4	21.1	0.7	4	22.8	0.5	2
6	23.5	0.7	3	22.7	0.8	3	24.4	0.6	1
7	24.4	0.9	2	24.3	1.0	2	-	-	-
8	25.0	1.2	1	25.6	1.4	1	-	-	-
Males									
1	11.0	0.3	6	8.3	0.4	6	9.8	0.4	5
2	15.2	0.3	6	12.9	0.4	6	14.3	0.4	5
3	17.7	0.3	5	15.7	0.4	5	17.5	0.4	4
4	19.3	0.3	4	17.8	0.4	4	19.5	0.4	3
5	20.6	0.3	3	19.5	0.4	3	20.9	0.4	2
6	21.5	0.3	2	20.9	0.5	2	22.0	0.6	1
7	21.8	0.4	1	21.4	0.7	1	-	-	-
8	-	-	-	-	-	-	-	-	-
Females									
1	12.0	0.3	6	8.7	0.4	6	10.8	0.3	4
2	16.9	0.3	6	14.0	0.5	6	15.8	0.4	4
3	19.8	0.3	6	17.7	0.6	6	19.5	0.4	4
4	22.1	0.3	5	20.3	0.6	5	21.9	0.3	3
5	24.0	0.4	4	22.5	0.7	4	24.3	0.4	2
6	25.5	0.4	3	24.3	0.7	3	26.1	0.4	1
7	26.2	0.4	2	25.9	0.6	2	-	-	-
8	26.7	0.6	1	26.9	0.7	1	-	-	-

Table 16.—von Bertalanffy parameters were simulated in FAST 2.1 using otoliths, dorsal spines, and scales for male and female walleye collected in Brookville Reservoir in 2009. K is the growth coefficient, t_0 is time when length is 0, r^2 is the correlation coefficient, length infinity (L_∞) was held constant at 27.0 in for male (M) and 32.0 in for female (F) walleye (R. Wisener, DFW, personal communication), and (I) is the number of iterations required to find an optimal solution. The von Bertalanffy equation was linearized to estimate the time t (years) required for each gender to reach preferred size (20.0 in) using age-length key mean length-at-capture, back-calculated mean length-at-capture, weighted mean back-calculated length, and un-weighted mean back-calculated length data.

	Otoliths		Dorsal Spines		Scales	
Parameter	M	F	M	F	M	F
FAST 2.1 Simulation: Age-length key mean length-at-capture						
K	0.180	0.189	0.155	0.163	0.214	0.206
t_0	-2.762	-1.985	-3.427	-2.810	-1.806	-1.695
r^2	0.936	0.977	0.885	0.971	0.921	0.962
L_∞	27.0	32.0	27.0	32.0	27.0	32.0
I	7	7	9	11	9	16
t	10.3	7.2	12.1	8.8	8.1	6.5
FAST 2.1 Simulation: Back-calculated mean length-at-capture						
K	0.174	0.200	0.168	0.190	0.251	0.218
t_0	-2.811	-1.550	-2.859	-1.791	-1.110	-1.387
r^2	0.910	0.974	0.926	0.992	0.972	0.972
L_∞	27.0	32.0	27.0	32.0	27.0	32.0
I	9	5	7	5	7	6
t	10.6	6.5	10.9	7.0	6.5	5.9
FAST 2.1 Simulation: Weighted mean back-calculated length						
K	0.192	0.213	0.209	0.227	0.261	0.247
t_0	-2.154	-1.296	-1.017	-0.530	-0.863	-0.669
r^2	0.940	0.991	0.975	0.997	0.992	0.998
L_∞	27.0	32.0	27.0	32.0	27.0	32.0
I	7	5	7	5	6	5
t	9.2	5.9	7.5	4.9	6.0	4.6
FAST 2.1 Simulation: Un-weighted mean back-calculated length						
K	0.213	0.213	0.220	0.222	0.263	0.253
t_0	-1.674	-1.376	-0.806	-0.506	-0.787	-0.648
r^2	0.975	0.989	0.991	0.998	0.994	0.999
L_∞	27.0	32.0	27.0	32.0	27.0	32.0
I	7	6	6	4	5	5
t	8.0	6.0	6.9	4.9	5.9	4.5

Table 17.—Annual mortality (*A*% ± 95% confidence intervals) estimates derived from Heincke's (H), Robson and Chapman's (RC), and catch-curve regressions (CC) for walleye (pooled-gender and separated by males and females) using otoliths, dorsal spines, and scales collected at Brookville Reservoir, 2009.

	Otoliths			Dorsal Spines			Scales		
	H	RC	CC	H	RC	CC	H	RC	CC
Pool-gender	55 ± 3	58 ± 2	62 ± 1	52 ± 3	58 ± 3	63 ± 2	68 ± 3	72 ± 2	83 ± 3
Males	41 ± 4	46 ± 3	57 ± 4	42 ± 4	52 ± 3	63 ± 4	48 ± 4	56 ± 3	53 ± 2
Females	64 ± 4	65 ± 3	62 ± 2	60 ± 4	63 ± 3	63 ± 2	75 ± 3	77 ± 3	81 ± 1

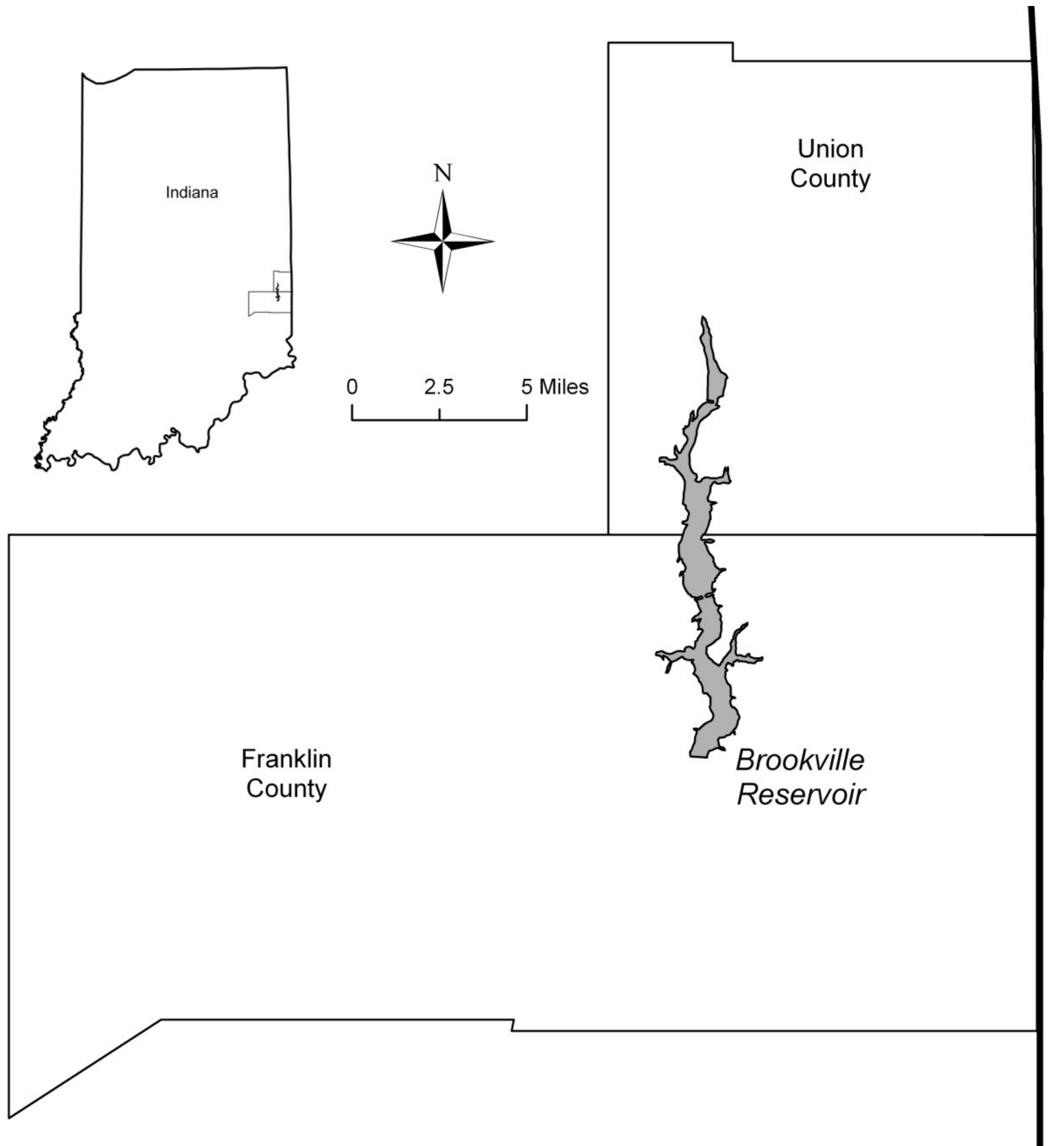

Figure 1.—Walleye were collected with gill nets along the dam of Brookville Reservoir from March 30 to April 8, 2009.

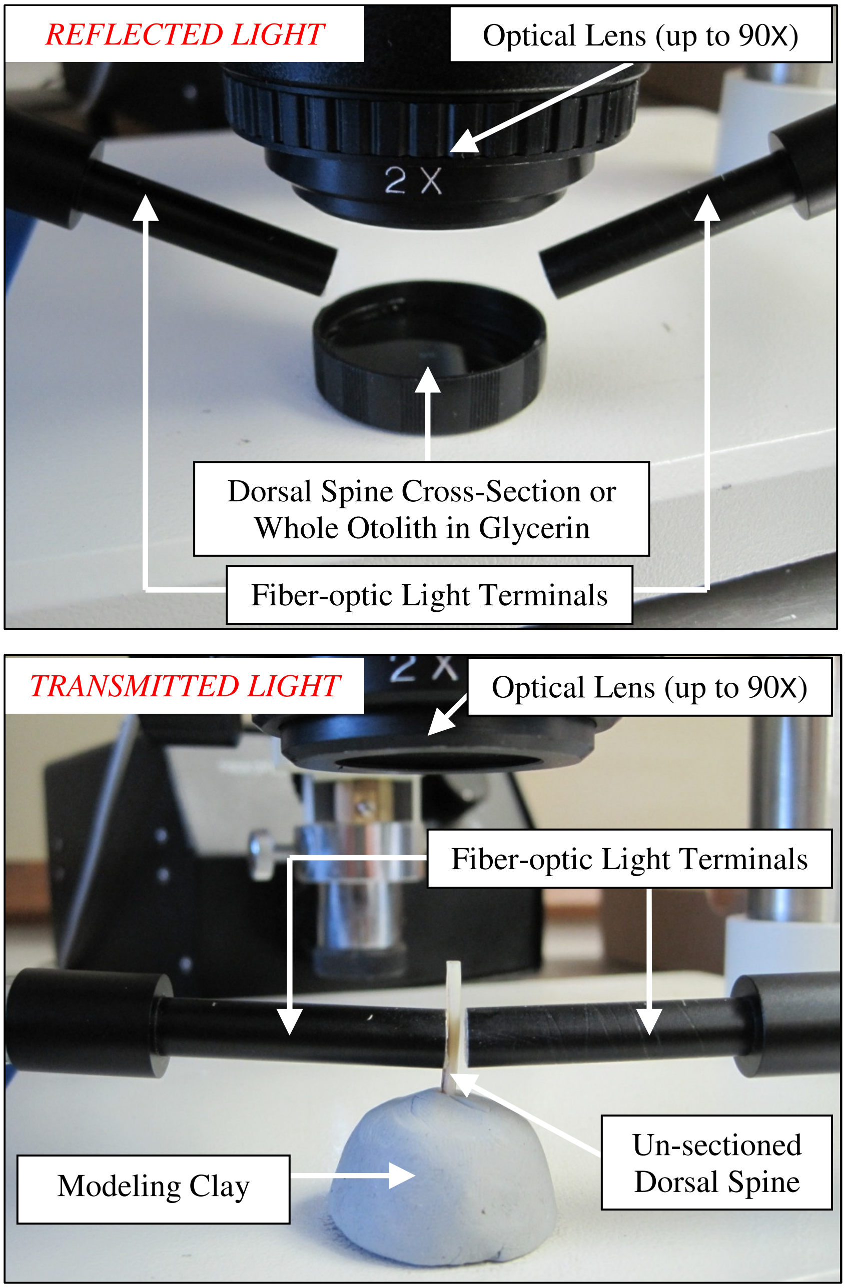

Figure 2.—The standard method (reflected light) for imaging walleye dorsal spine cross-sections and whole otoliths (above); and the alternative method (transmitted light) for imaging un-sectioned walleye dorsal spines (below).

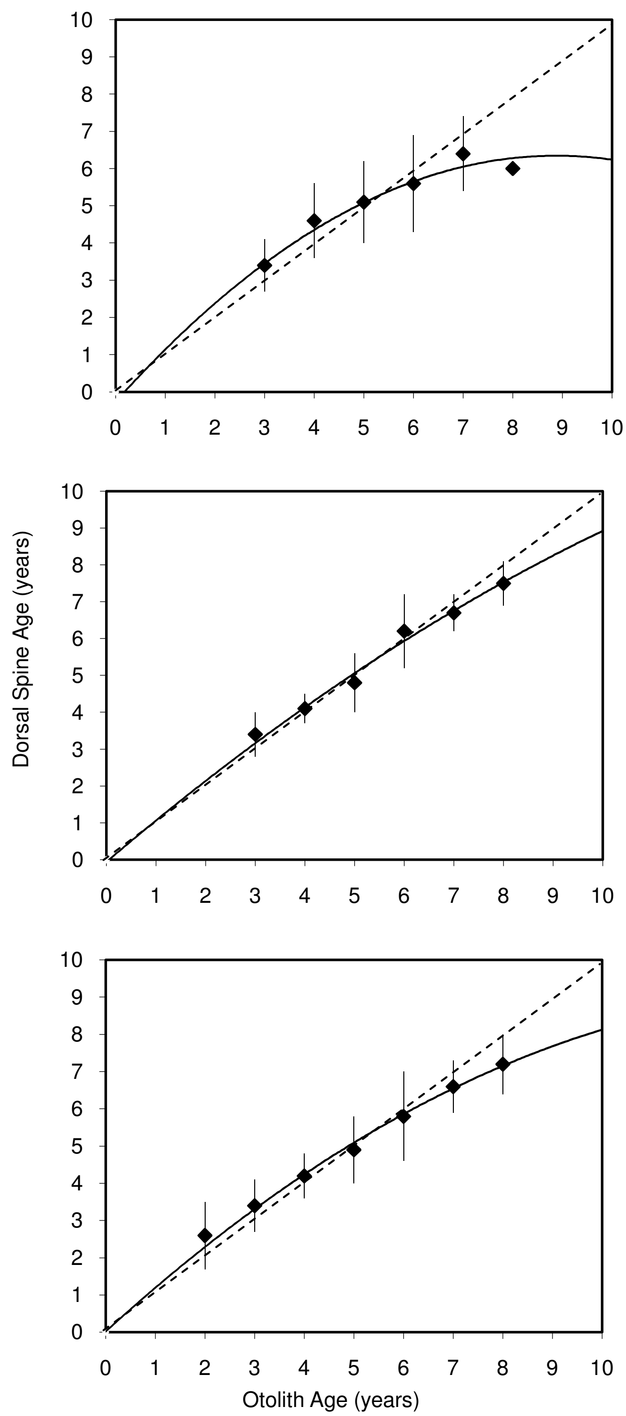

Figure 3.—Age-bias plots illustrating mean (± SD) age cross comparisons between otoliths (*x*-axes) and dorsal spines (*y*-axes) for male (above), female (middle), and all genders combined (below) walleye that were collected in Brookville Reservoir, 2009. The solid lines represent bias-curves and the dashed lines represent theoretical 1:1 equivalence.

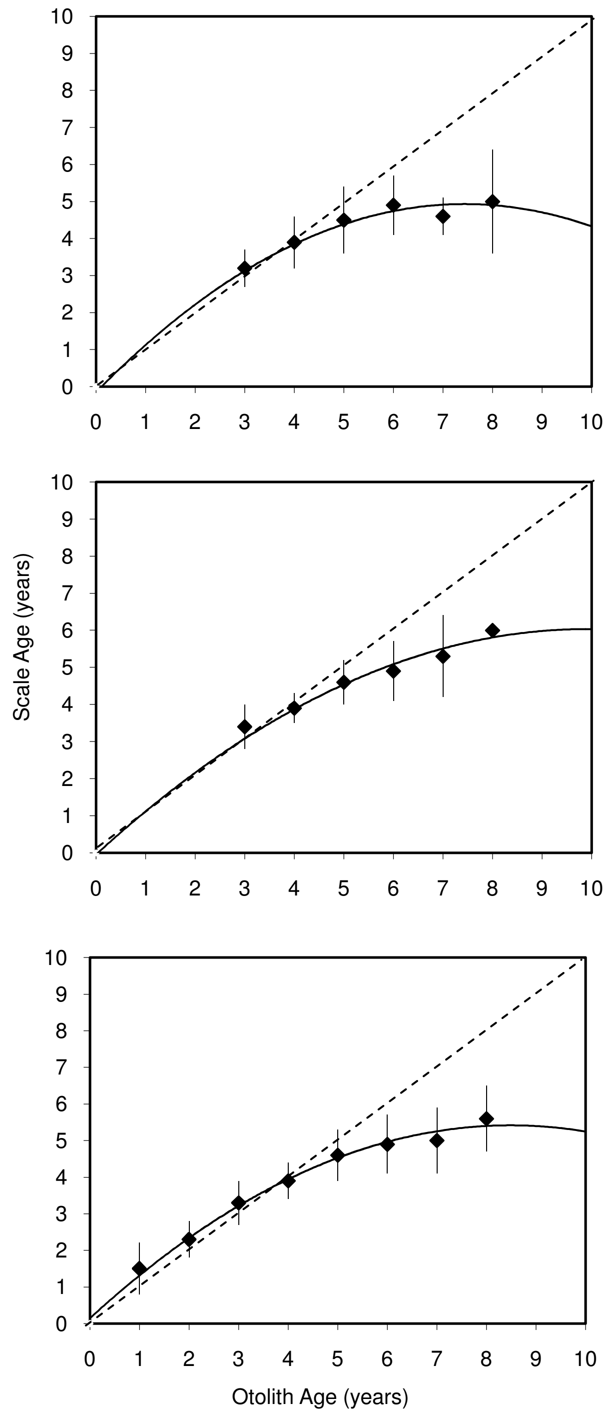

Figure 4.— Age-bias plots illustrating mean (± SD) age cross comparisons between otoliths (*x*-axes) and scales (*y*-axes) for male (above), female (middle), and all genders combined (below) walleye that were collected in Brookville Reservoir, 2009. The solid lines represent bias-curves and the dashed lines represent theoretical 1:1 equivalence.

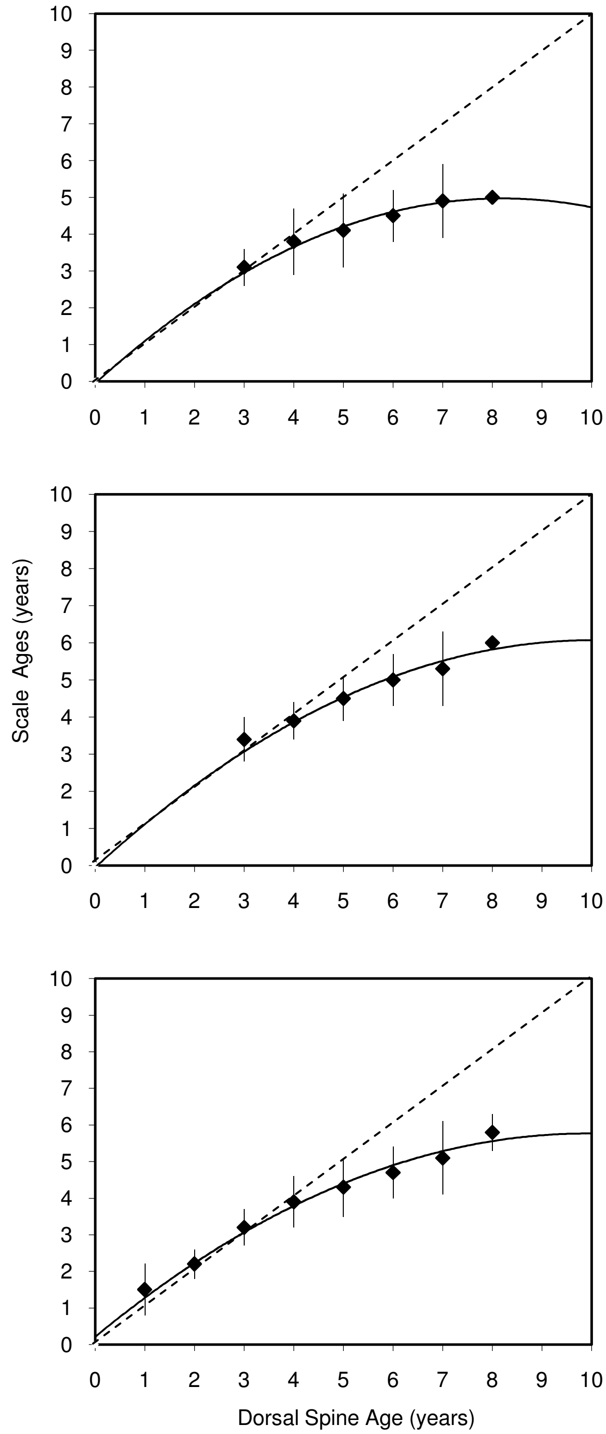

Figure 5.— Age-bias plots illustrating mean (± SD) age cross comparisons between dorsal spines (*x*-axes) and scales (*y*-axes) for male (above), female (middle), and all genders combined (below) walleye that were collected in Brookville Reservoir, 2009. The solid lines represent bias-curves and the dashed lines represent theoretical 1:1 equivalence.

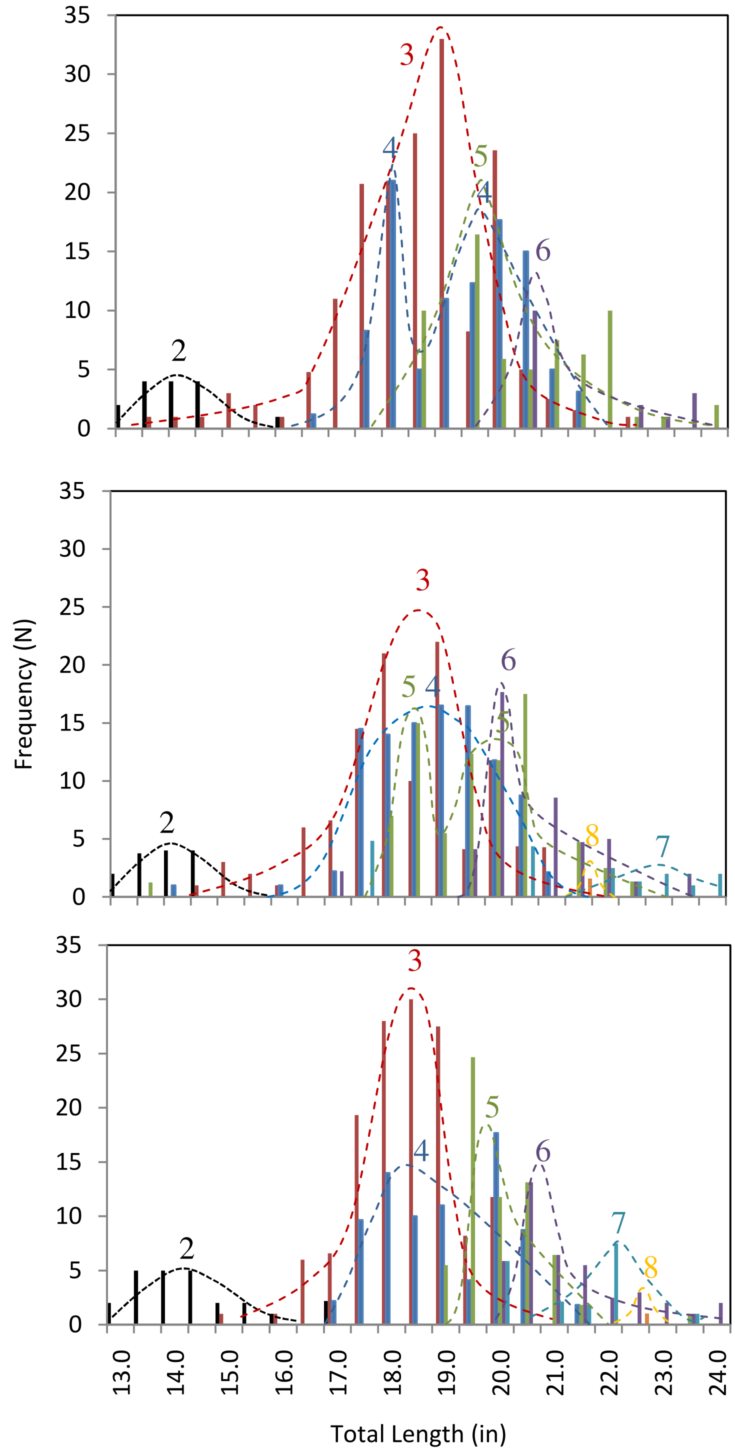

Figure 6.— Post-concert age-length distributions for male walleye derived by applying age-length keys based on scales (above), dorsal spines (middle), and otoliths (below) to the length frequency distribution of fish captured during broodstock operations at Brookville Reservoir, 2009. Age-specific distributions are shown at the peak of the bell-curves.

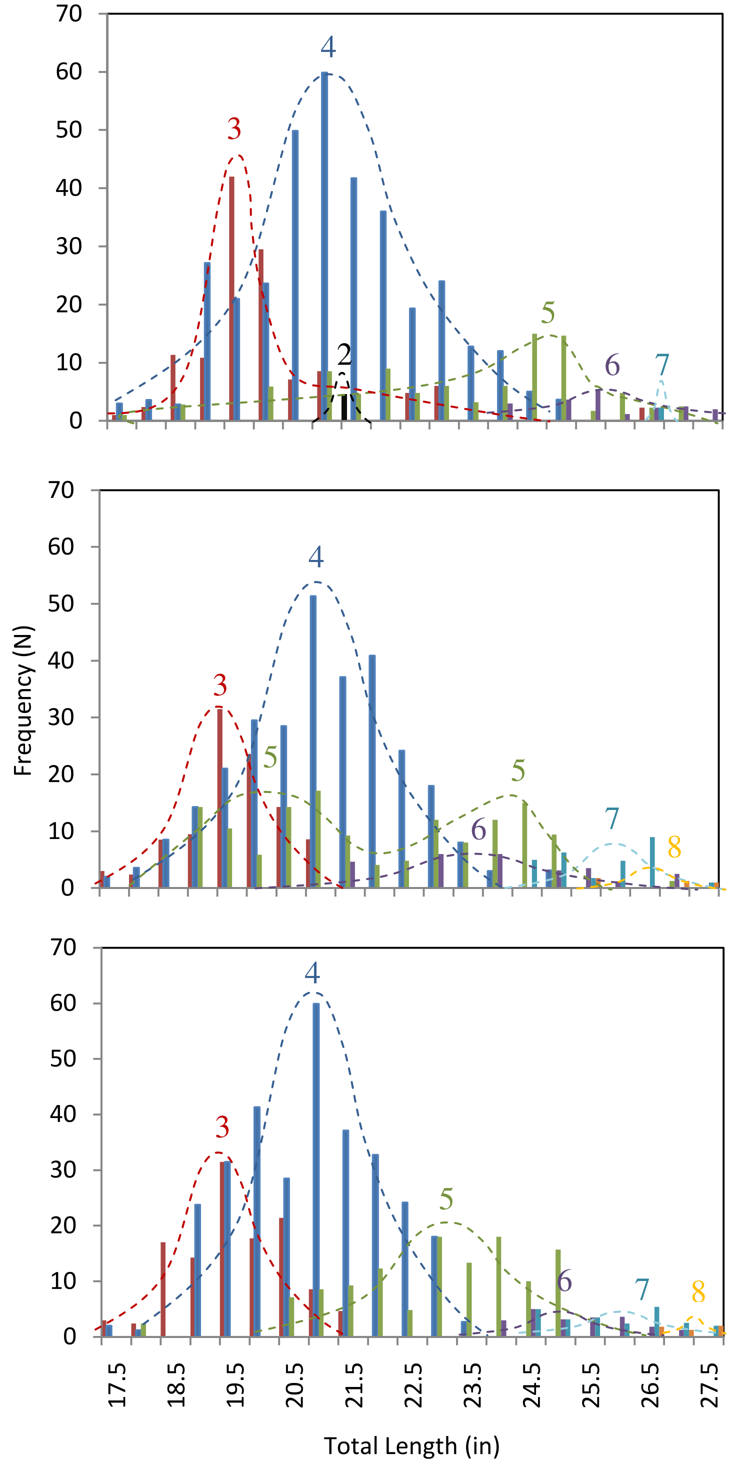

Figure 7.—Post-concert age-length distributions for female walleye derived by applying age-length keys based on scales (above), dorsal spines (middle), and otoliths (below) to the length frequency distribution of fish captured during broodstock operations at Brookville Reservoir, 2009. Age-specific distributions are shown at the peak of the bell-curves.

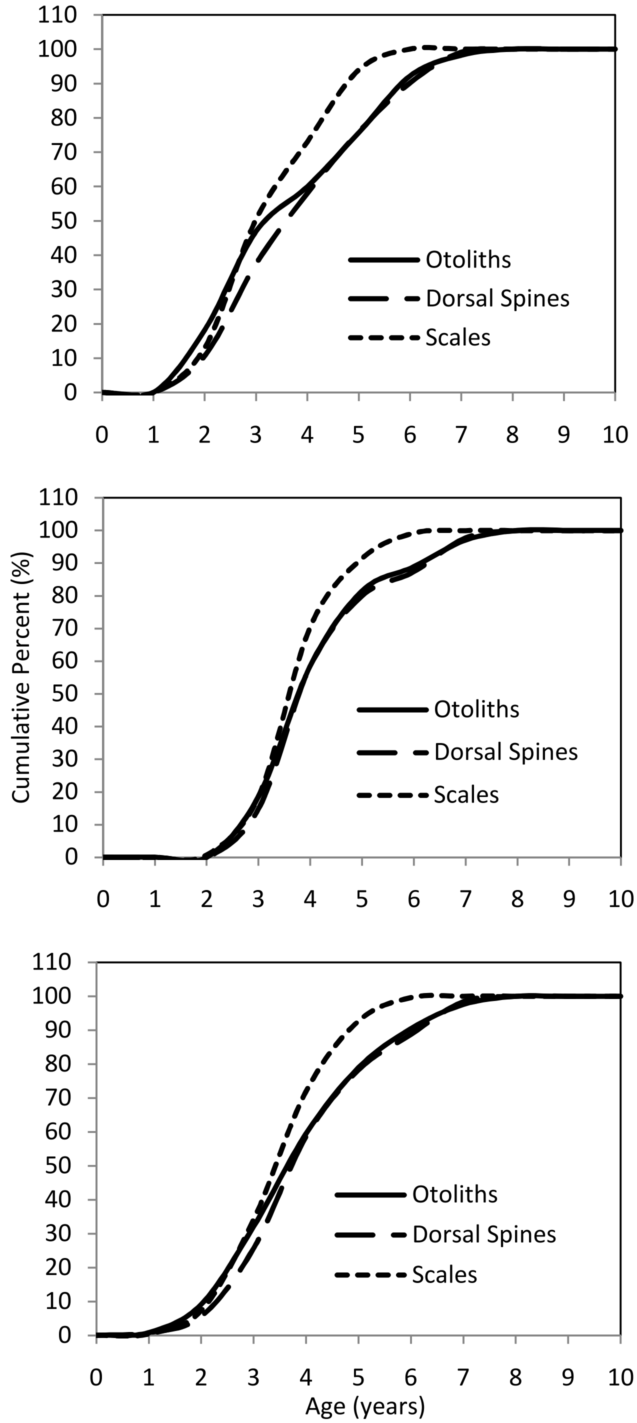

Figure 8.—Cumulative age distribution comparisons for male (above), female (middle), and pooled-gender (below) walleye that were collected in Brookville Reservoir, 2009. Kolmogorov-Smirnov pairwise comparisons among calcified structures and gender are summarized in Table 11.

Figure 9a.—Digital image of a scale for walleye 027 (Male, 22.1 in, 3.7 lb) collected in Brookville Reservoir, 2009. Initial consensus marks among three age-analysts suggested walleye 027 was age-4, which was inconsistent with age assigned to the dorsal spine (age-6; Figures 9b and 9c) and otolith (age-6; Figure 9d). When this scale was re-analyzed after being directly compared with the dorsal spine and otolith, it was determined that age-3 and age-5 (dashed-arrows) were likely missed during the concert read. Overall, scales were generally under-aged when compared to dorsal spines and otoliths.

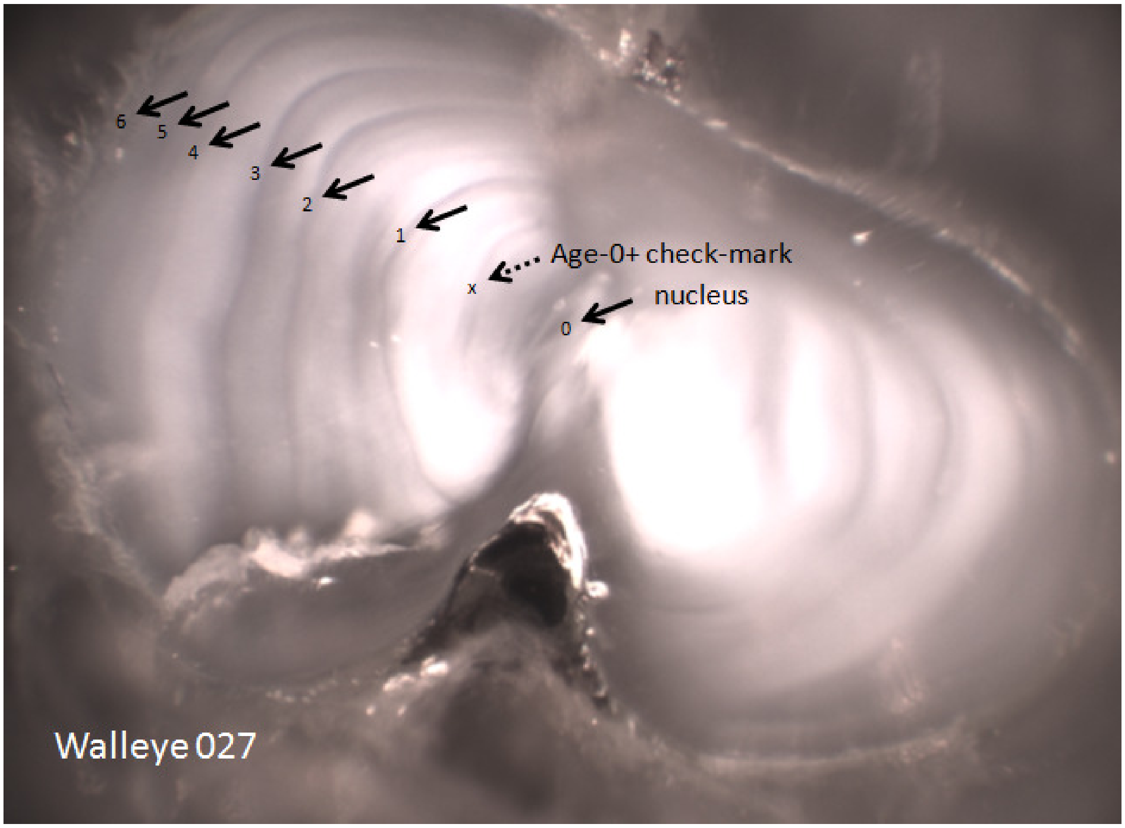

Figure 9b.—Digital image of a dorsal spine using the standard method (i.e., reflected light, 75 µm cross-section) for walleye 027 (Male, 22.1 in, 3.7 lb) collected in Brookville Reservoir, 2009. Initial consensus marks among three age-analysts suggested walleye 027 was age-6, which was inconsistent with the age assigned to the scale (age-4; Figure 9a) and consistent with the age assigned to the otolith (age-6; Figure 9d). When this dorsal spine was re-analyzed after being directly compared with the scale and otolith, it was determined that age-x was likely the age-0+ check-mark frequently observed among other dorsal spines. Overall, dorsal spines were more difficult to interpret than otoliths but were also more consistent than scales.

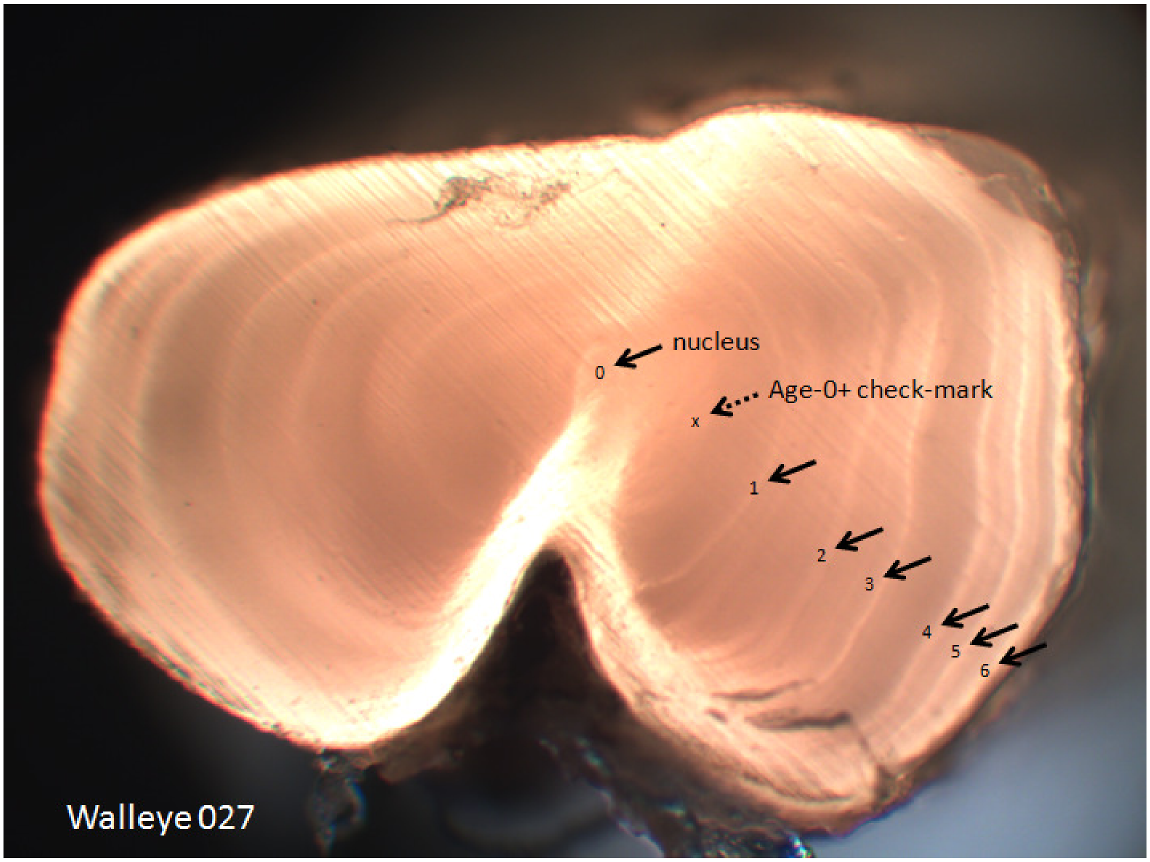

Figure 9c.—Digital image of a dorsal spine using the alternative method (i.e., transmitted light, un-sectioned) for walleye 027 (Male, 22.1 in, 3.7 lb) collected in Brookville Reservoir, 2009. Initial consensus marks among three age-analysts suggested walleye 027 was age-6, which was inconsistent with the ages assigned to the scale (age-4; Figure 9a) and consistent with the age assigned to the otolith (age-6; Figure 9d). Notice that the same age-0+ check-mark is evident for the alternative method when compared to the standard method (i.e., reflected light, 75 µm cross-section; Figure 9b). The differences between the two methods are that the color patterns that define the annuli and growth periods are reversed (i.e., annuli are light rather than dark, and growth is dark rather than light). One advantage to the alternative method is that the spines do not have to be sectioned by a low speed saw. One disadvantage to the alternative method is that it is often time-consuming to fine-tune the fiber-optic terminals in order to sufficiently transmit the light through the spine and capture a clear digital image.

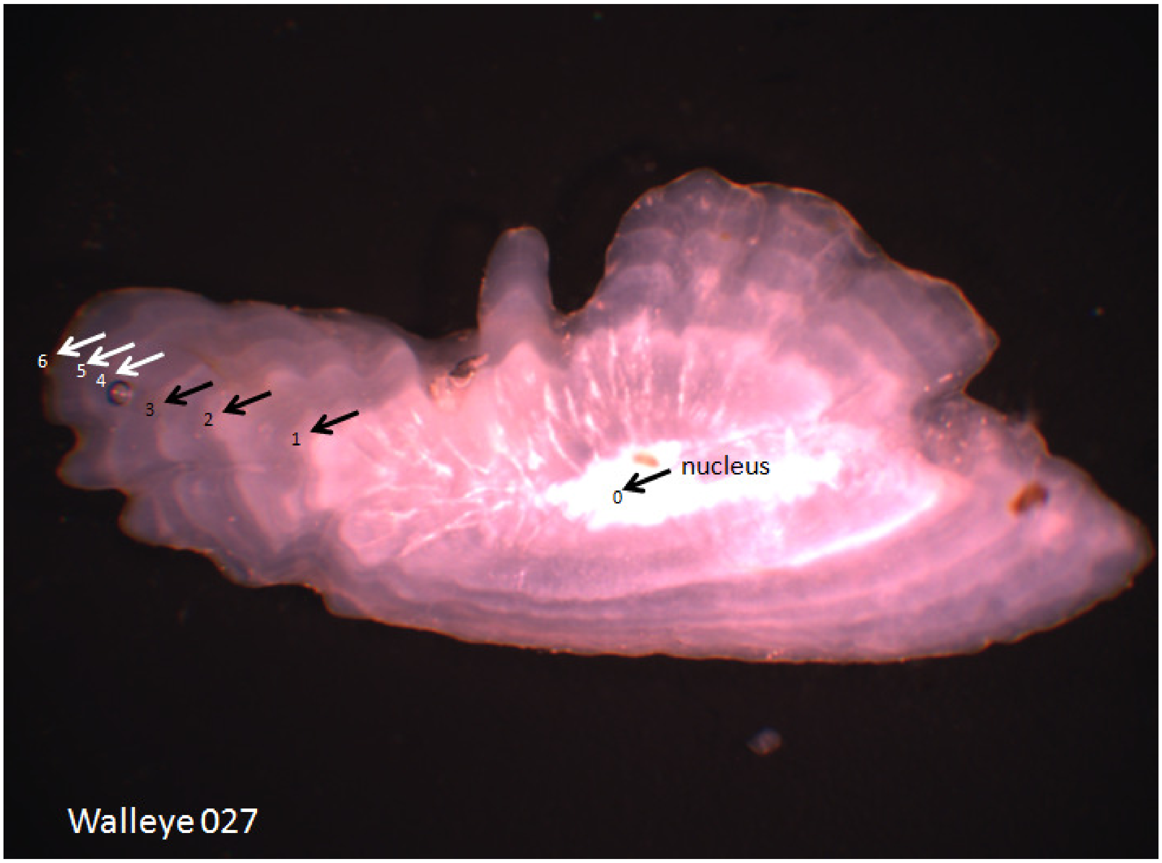

Figure 9d.—Digital image of a whole otolith for walleye 027 (Male, 22.1 in, 3.7 lb) collected in Brookville Reservoir, 2009. Initial consensus marks among three age-analysts suggested walleye 027 was age-6, which was inconsistent with age assigned to the scale (age-4; Figure 9a) and consistent with the age assigned to the dorsal spine (age-6; Figures 9b and 9c). Otoliths were the most unbiased and precise aging structure among three age-analysts and thus the best available surrogate for known-age fish.

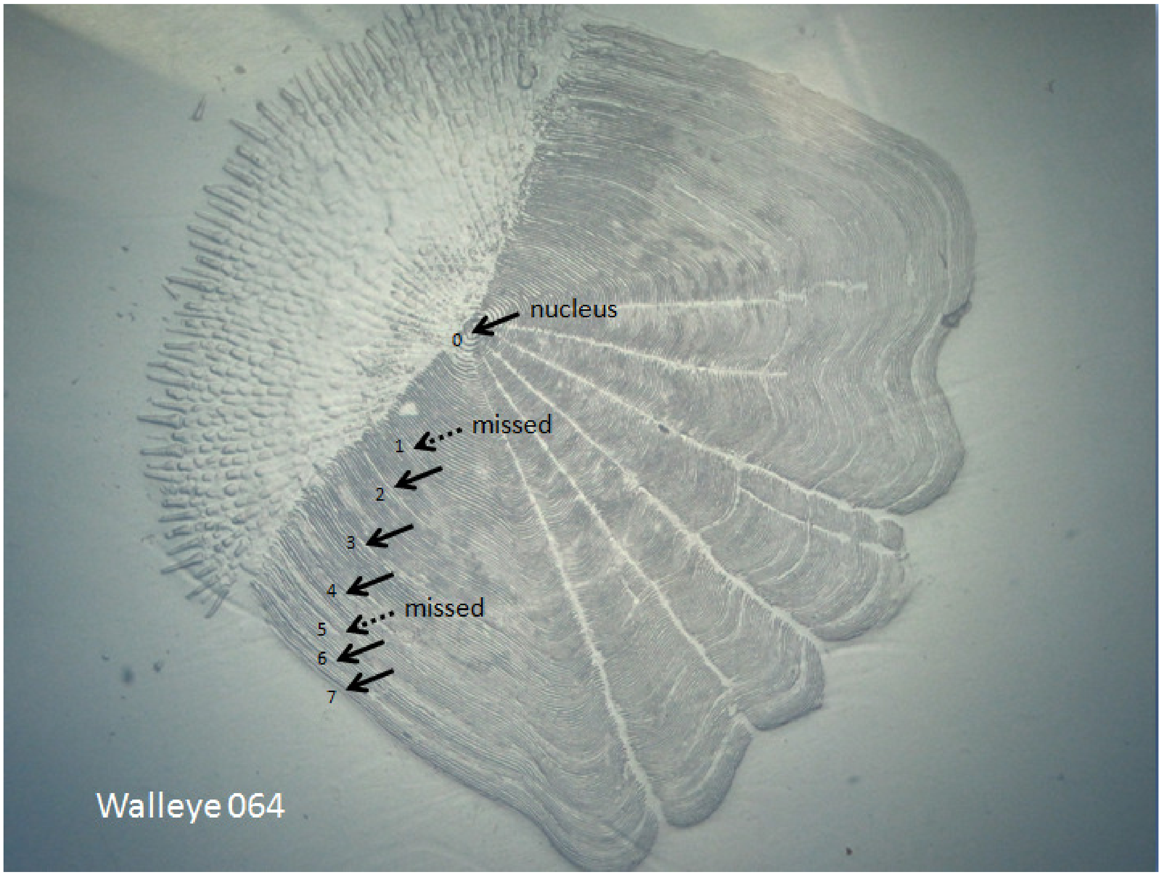

Figure 10a.—Digital image of a scale for walleye 064 (Female, 26.1 in, 6.8 lb) collected in Brookville Reservoir, 2009. Initial consensus marks among three age-analysts suggested walleye 064 was age-5, which was inconsistent with the ages assigned to the dorsal spine (age-7; Figures 10b and 10c) and otolith (age-7; Figure 10d). When this scale was re-analyzed after being directly compared with the dorsal spine and otolith, it was determined that age-1 and age-5 (dashed-arrows) were likely missed during the concert read. Overall, scales were generally under-aged when compared to dorsal spines and otoliths.

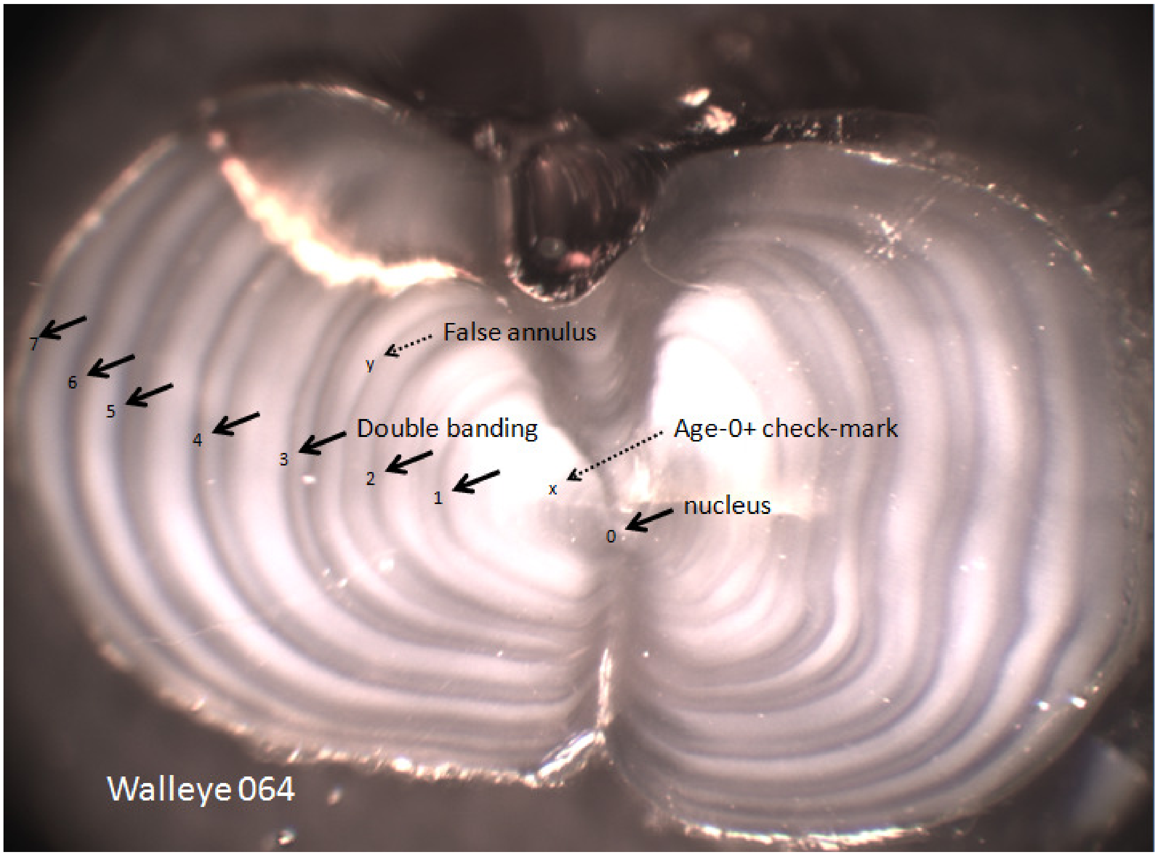

Figure 10b.—Digital image of a dorsal spine using the standard method (i.e., reflected light, 75 µm cross-section) for walleye 064 (Female, 26.1 in, 6.8 lb) collected in Brookville Reservoir, 2009. Initial consensus marks of the dorsal spine among three age-analysts suggested walleye 064 was age-7, which was inconsistent with age assigned to the scale (age-5; Figure 10a), but consistent with the otolith (age-7; Figure 10d). Dorsal spines frequently displayed: (1) an age-0+ check-mark (age-x) that can easily be misinterpreted as the first annulus; (2) false annuli (age-y); and (3) double-banding (ages 3, 4 and 5). Overall, dorsal spines were more difficult to interpret than otoliths but were also more consistent than scales.

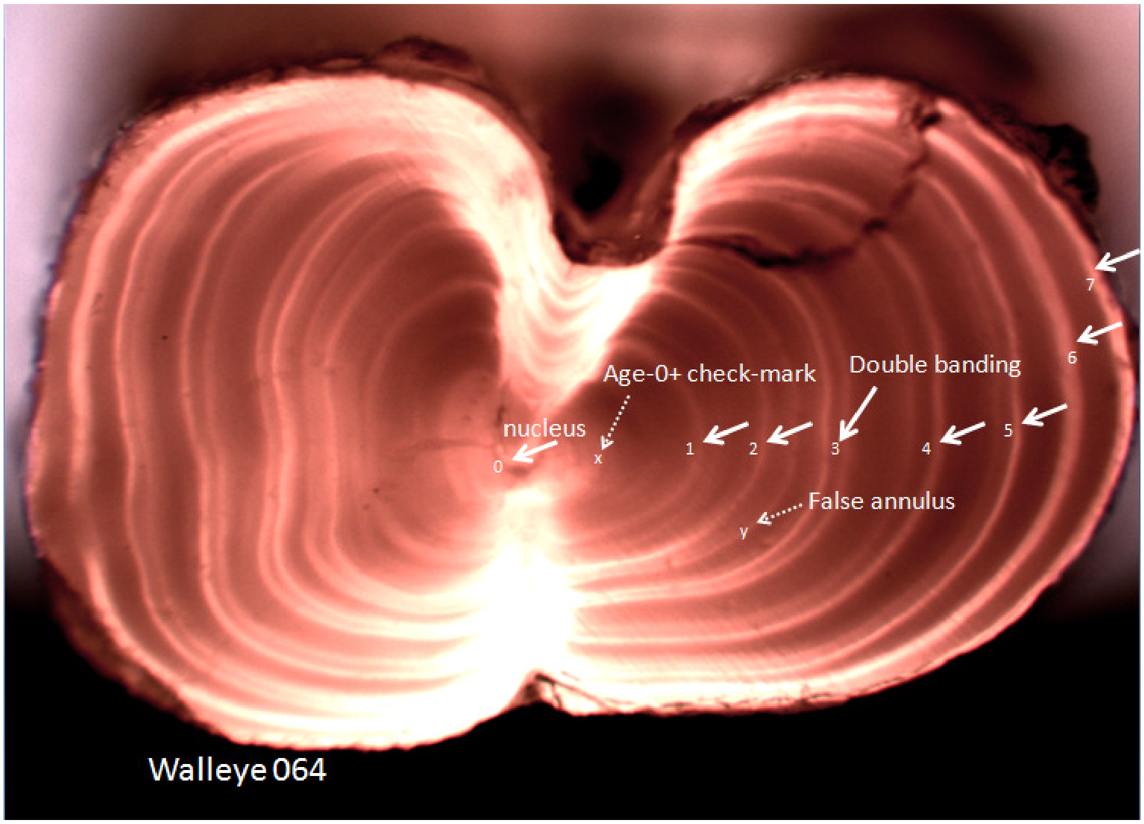

Figure 10c.—Digital image of a dorsal spine using the alternative method (i.e., transmitted light, un-sectioned) for walleye 064 (Female, 26.1 in, 6.8 lb) collected in Brookville Reservoir, 2009. Notice that the same features are evident for the alternative method (i.e., age-0+ check-mark, false annulus, and double-banding) when compared to the standard method (i.e., reflected light, 75 µm cross-section; Figure 10b).

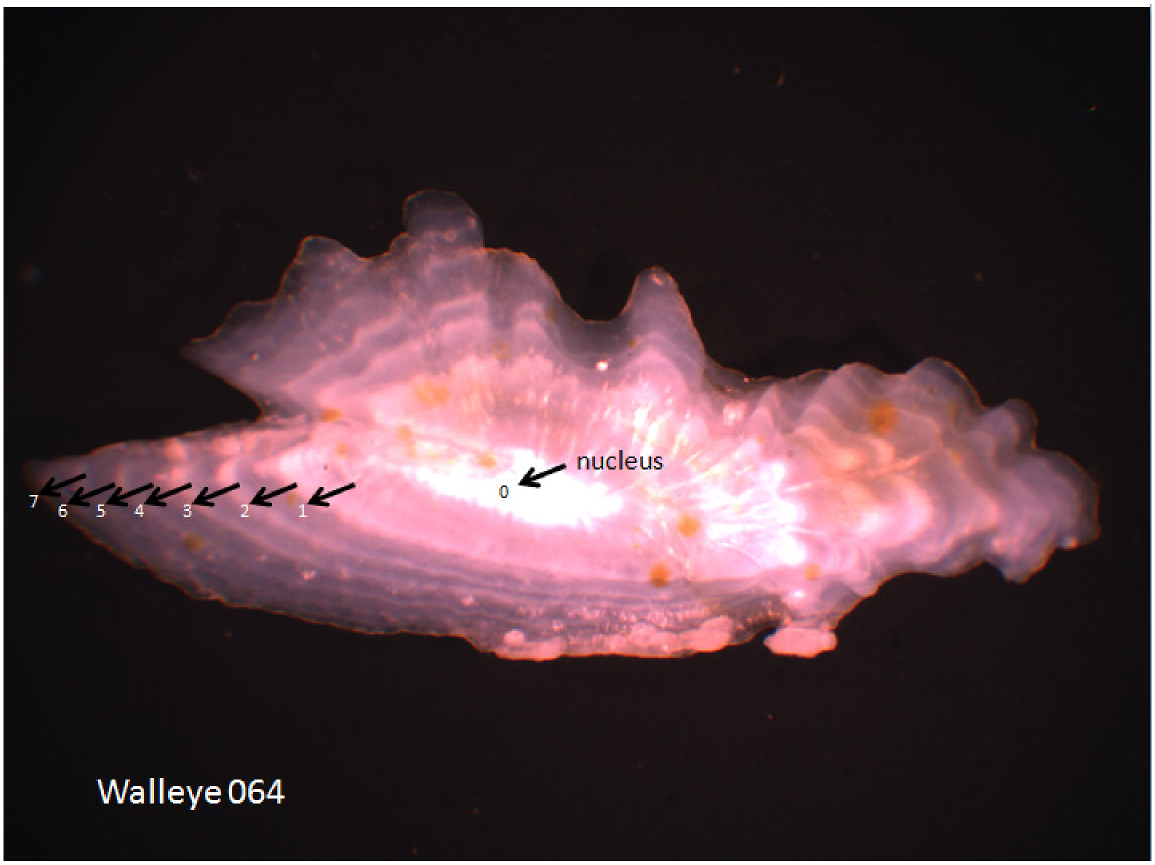

Figure 10d.—Digital image of a whole otolith for walleye 064 (Female, 26.1 in, 6.8 lb) collected in Brookville Reservoir, 2009. Initial consensus marks among three age-analysts suggested walleye 064 was age-7, which was inconsistent with age assigned to the scale (age-5; Figure 10a), but consistent with the dorsal spine (age-7; Figures 10b and 10c). Otoliths were the most unbiased and precise aging structure among three age-analysts and thus the best available surrogate for known-age fish.

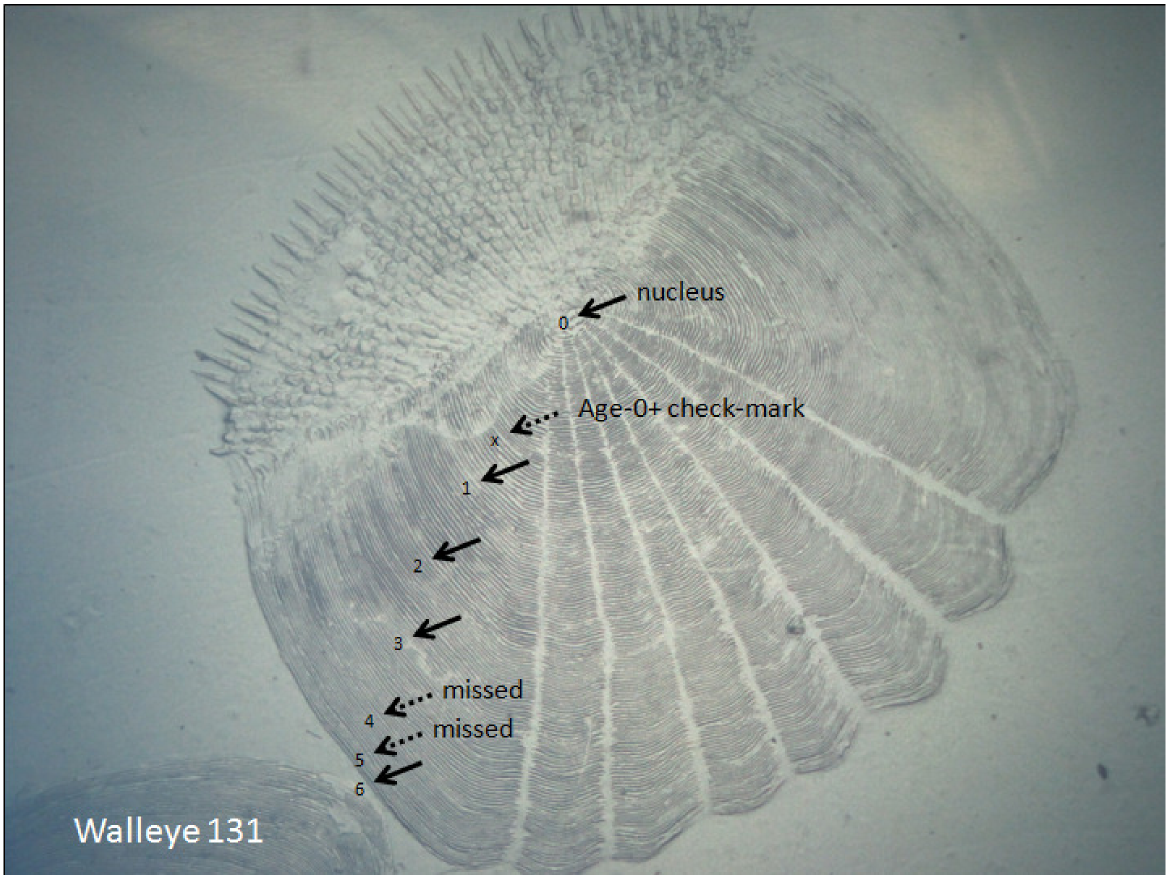

Figure 11a.—Digital image of a scale for walleye 131 (Male, 23.2 in, 4.7 lb) collected in Brookville Reservoir, 2009. Initial consensus marks among three age-analysts suggested walleye 131 was age-5, which was inconsistent with age assigned to the dorsal spine (age-7; Figures 11b and 11c) and the otolith (age-6; Figure 11d). When this scale was re-analyzed after being directly compared with the dorsal spine and otolith, it was determined that age-x was incorrectly marked and determined to be an age-0+ check-mark. Age-4, and age-5 (dashed-arrows) were likely missed during the concert read. Overall, scales were generally under-aged when compared to dorsal spines and otoliths.

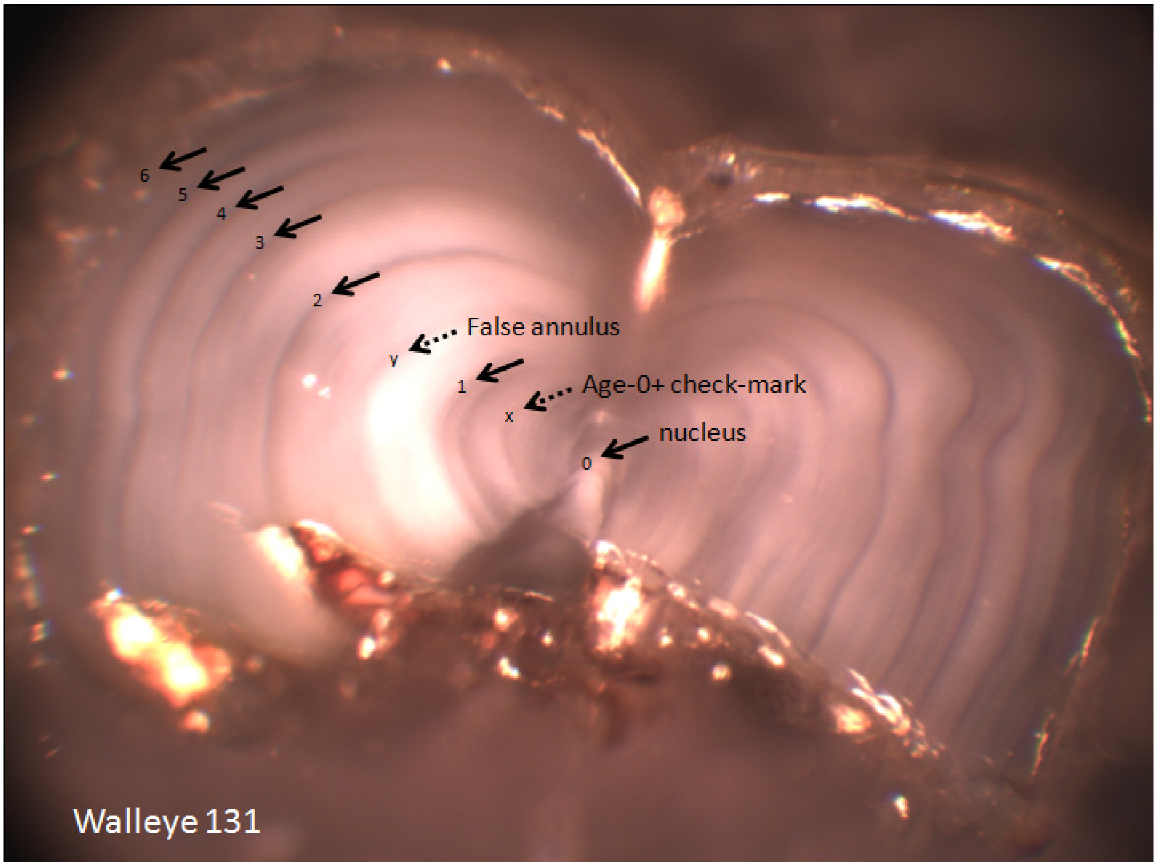

Figure 11b.—Digital image of a dorsal spine using the standard method (i.e., reflected light, 75 µm cross-section) for walleye 131 (Male, 23.2 in, 4.7 lb) collected in Brookville Reservoir, 2009. Initial consensus marks among three age-analysts suggested walleye 131 was age-7, which was inconsistent with the age assigned to the scale (age-5; Figure 11a) and the otolith (age-6; Figure 11d). When this dorsal spine was re-analyzed after being directly compared with the scale and otolith, it was determined that age-x was likely the age-0+ check-mark frequently observed among other dorsal spines. One annulus that was initially marked was later identified as a false annulus (age-y) because the strength of the pattern was weaker than observed among true annuli. Overall, dorsal spines were more difficult to interpret than otoliths but were also more consistent than scales.

Figure 11c.—Digital image of a dorsal spine using the alternative method (i.e., transmitted light, un-sectioned) for walleye 131 (Male, 23.2 in, 4.7 lb) collected in Brookville Reservoir, 2009. Initial consensus marks among three age-analysts suggested walleye 131 was age-7, which was inconsistent with the ages assigned to the scale (age-5; Figure 11a) and otolith (age-6; Figure 11d). Notice that the same features are evident for the alternative method (i.e., age-0+ check-mark [age-x] and false annuli [age-y]) when compared to the standard method (i.e., reflected light, 75 µm cross-section; Figure 11b).

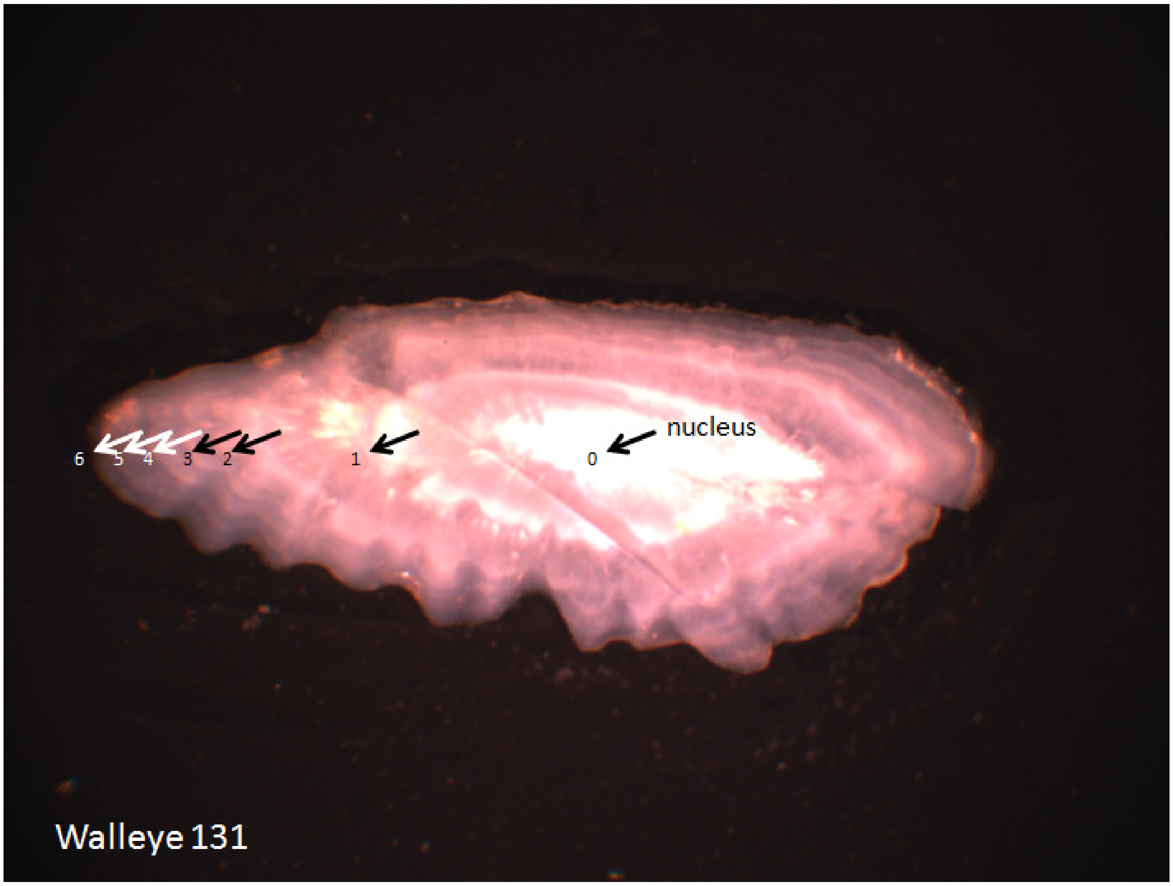

Figure 11d.—Digital image of a whole otolith for walleye 131 (Male, 23.2 in, 4.7 lb) collected in Brookville Reservoir, 2009. Initial consensus marks among three age-analysts suggested walleye 131 was age-6, which was inconsistent with ages assigned to the scale (age-5; Figure 11a) and dorsal spine (age-7; Figures 11b and 11c). Otoliths were the most unbiased and precise aging structure among three age-analysts and thus the best available surrogate for known-age fish.

Figure 12a.—Digital image of a scale for walleye 215 (Female, 20.5 in, 2.9 lb) collected in Brookville Reservoir, 2009. Initial consensus marks among three age-analysts suggested walleye 215 was age-4, which was inconsistent with age assigned to the dorsal spine (age-5; Figures 12b and 12c) and otolith (age-3; Figure 12d). When this scale was re-analyzed after being directly compared with the dorsal spine and otolith, it was determined that age-x was actually a false annulus because it did not show the strong cross-over pattern observed among true annuli. Overall, scales were generally under-aged when compared to dorsal spines and otoliths.

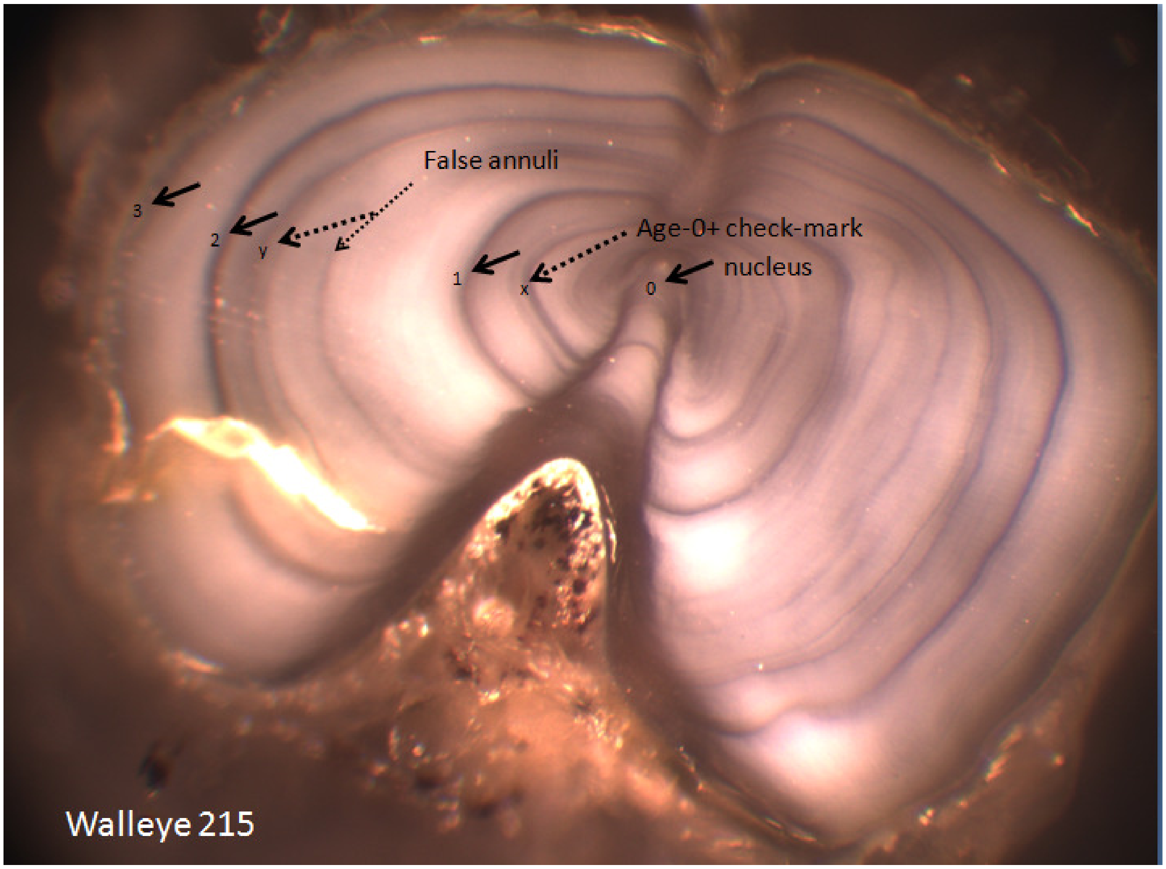

Figure 12b.—Digital image of a dorsal spine using the standard method (i.e., reflected light, 75 µm cross-section) for walleye 215 (Female, 20.5 in, 2.9 lb) collected in Brookville Reservoir, 2009. Initial consensus marks among three age-analysts suggested walleye 215 was age-5, which was inconsistent with the ages assigned to the scale (age-4; Figure 12a) and otolith (age-3; Figure 12d). When this dorsal spine was re-analyzed after being directly compared with the scale and otolith, it was determined that initial mark age-x was likely the age-0+ check-mark frequently observed among other dorsal spines. One false annulus was misinterpreted (age-y) and one was correctly identified (between age-1 and false annulus age-y) because the strength of the patterns were weaker than observed among true annuli. Overall, dorsal spines were more difficult to interpret than otoliths but were also more consistent than scales.

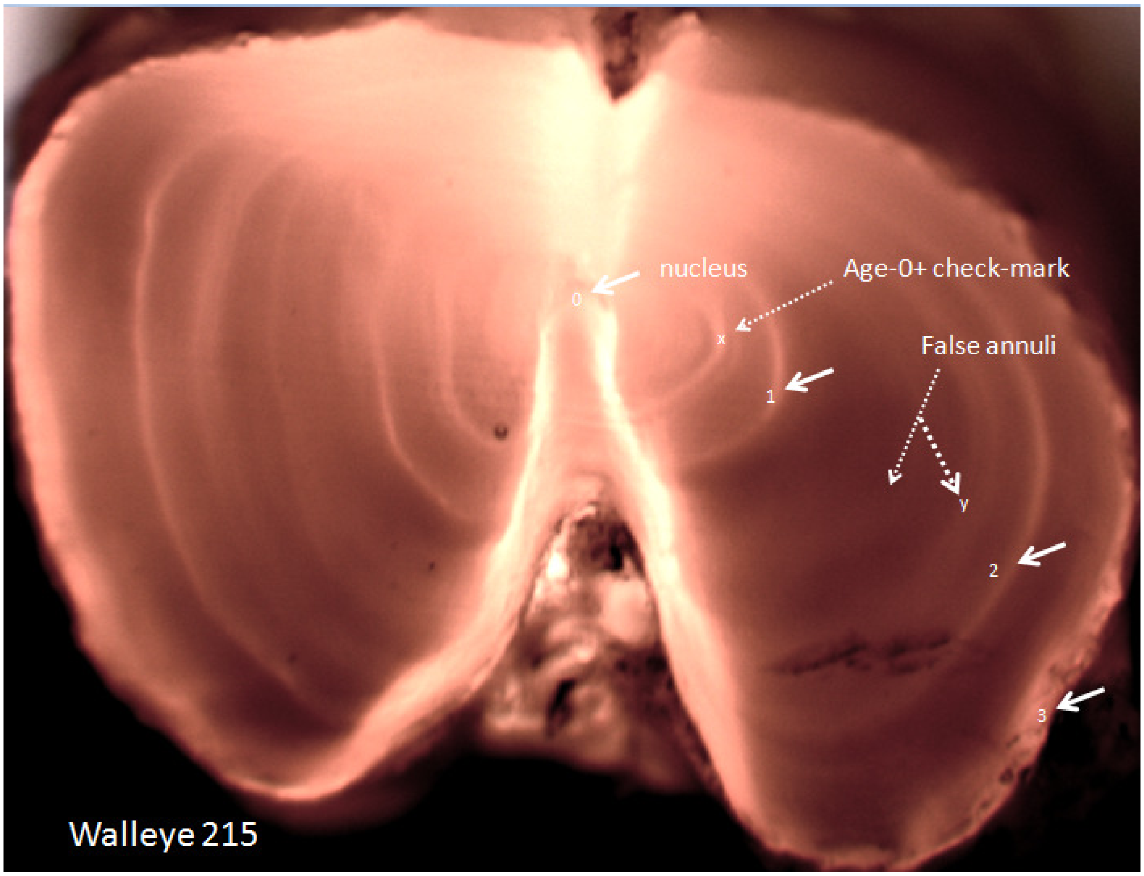

Figure 12c.—Digital image of a dorsal spine using the alternative method (i.e., transmitted light, un-sectioned) for walleye 215 (Female, 20.5 in, 2.9 lb) collected in Brookville Reservoir, 2009. Initial consensus marks among three age-analysts suggested walleye 215 was age-5, which was inconsistent with the ages assigned to the scale (age-4; Figure 12a) and otolith (age-3; Figure 12d). Notice that the same features are evident for the alternative method (i.e., age-0+ check-mark [age-x] and false annuli [age-y]) when compared to the standard method (i.e., reflected light, 75 µm cross-section; Figure 12b).

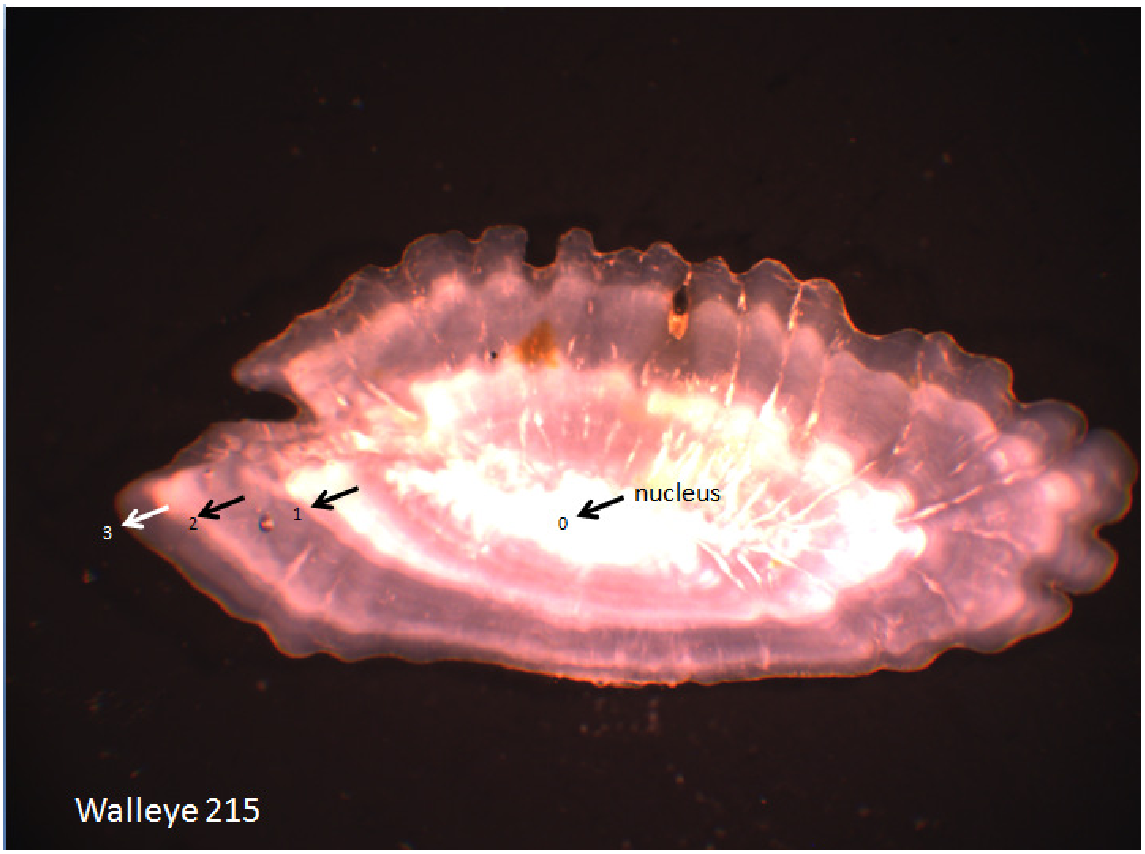

Figure 12d.—Digital image of a whole otolith for walleye 215 (Female, 20.5 in, 2.9 lb) collected in Brookville Reservoir, 2009. Initial consensus marks among three age-analysts suggested walleye 215 was age-3, which was inconsistent with ages assigned to the scale (age-4; Figure 12a) and dorsal spine (age-5; Figures 12b and 12c). Otoliths were the most unbiased and precise aging structure among three age-analysts and thus the best available surrogate for known-age fish.